

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant	: George J. MURAKAWA et al.		
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TC/A.U.	: 1637		
Examiner	: Suryaprabha Chunduru		
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Commissioner of Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**APPELLANTS' BRIEF ON APPEAL UNDER 37 C.F.R. § 41.37**

Dear Sir:

The following comprises the Appellants' Brief on Appeal from the final rejection, dated December 23, 2008, of claims 190-234 and 242-255. This Brief is accompanied by a Request for Extension of time of five months to extend the date of the June 23, 2009 Notice of Appeal to January 25, 2010 and is thus timely filed.

**I. REAL PARTY IN INTEREST**

The real party of interest is City of Hope.

**II. RELATED APPEALS AND INTERFERENCES**

The present application was involved in a prior appeal of a final rejection, Appeal No. 93-4108. In a Remand to Examiner (Paper No. 32 of the present application) dated August 8, 1996 in that appeal, the Board of Patent Appeals and Interferences (hereinafter "the Board") returned the application to the Examiner for further action. The present application was also involved in a prior interference, Patent Interference No. 105,055. The Board granted party Wang's preliminary motion 1 in a Memorandum Opinion and Order dated November 5, 2003

(Paper No. 49 of the present application<sup>1</sup>). The Board denied party Murakawa's preliminary motion 1 in a Memorandum Opinion and Order dated April 5, 2004 (Paper No. 50 of the present application<sup>2</sup>) and issued a Final Judgment against party Murakawa on April 5, 2004 (Paper No. 51 of the present application<sup>3</sup>) as to party Murakawa claims corresponding to Counts 1 and 2. The Board also issued a Decision on Rehearing on April 29, 2004 (Paper No. 50 of the Interference No. 105,055).

There are no other pending appeals, interferences or judicial proceedings known to Appellants, the Appellants' legal representative, or assignee which may be related to, directly affect or be directly affected by, or have a bearing on the Board's decision in the pending appeal.

### **III. STATUS OF CLAIMS**

Claims 190-225, 242-245 and 249-255 are pending and are rejected. Claims 1-189, 226-241 and 246-248 have been cancelled.

### **IV. STATUS OF AMENDMENTS**

On May 29, 2009, Applicants filed an Amendment After Final in which claims 226-234 and 246-248 were canceled. In an Advisory Action dated June 16, 2009, the Examiner indicated that the proposed amendments would be entered for purposes of appeal. The Claims Appendix below reflects the cancellation of claims 226-234 and 246-248.

### **V. SUMMARY OF CLAIMED SUBJECT MATTER**

#### ***Independent Claim 190***

The subject matter of claim 190 embodies a process for the amplification of a target viral RNA and a reference RNA in a sample (page 2, lines 2-13; page 4, lines 1-9). The process comprises (i) selecting a sequence present in the target viral RNA (page 4, line 22 – page 5, line

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<sup>1</sup> Paper No. 36 of Interference No. 105,055.

<sup>2</sup> Paper No. 47 of Interference No. 105,055.

<sup>3</sup> Paper No. 48 of Interference No. 105,055.

9; page 5, lines 10-25), (ii) adding a known quantity of a reference RNA sequence to the sample (page 7, lines 1-6 and 15-19), (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19) and (iv) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

### ***Dependent Claims 193 196 and 198***

Claim 193 depends directly from claim 190. Claim 193 recites that a primer used in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding site (page 10, lines 24-29; original claim 23).

Claim 196 depends indirectly from claim 190 via intermediate claims 194 and 195. Claim 196 recites that the label on the probes used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

Claim 198 depends indirectly from claim 190 via intermediate claims 194 and 197. Claim 198 recites that the label on the primers used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

***Independent Claim 199***

The subject matter of claim 199 embodies a process for the amplification of a target viral RNA and a reference RNA in a sample (page 2, lines 2-13; page 4, lines 1-9). The process comprises (i) selecting a sequence present in the target viral RNA (page 4, line 22 – page 5, line 9; page 5, lines 10-25), (ii) adding a known quantity of a reference RNA sequence to the sample (page 7, lines 1-6 and 15-19), (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction (page 2, lines 7-8; page 8, line 26 – page 9, line 5; page 12, line 30-32) and then to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19) and (iv) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

***Dependent Claims 202, 205 and 207***

Claim 202 depends directly from claim 199. Claim 202 recites that a primer used in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding site (page 10, lines 24-29; original claim 23).

Claim 205 depends indirectly from claim 199 via intermediate claims 203 and 204. Claim 205 recites that the label on the probes used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

Claim 207 depends indirectly from claim 199 via intermediate claims 203 and 206. Claim 207 recites that the label on the primers used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

### ***Independent Claim 208***

The subject matter of claim 208 embodies a process for the amplification of a target viral RNA and a reference RNA in a sample (page 2, lines 2-13; page 4, lines 1-9). The process comprises (i) combining a known quantity of a reference RNA sequence with the sample (page 7, lines 1-6 and 15-19), (ii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19) and (iii) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

### ***Dependent Claims 211, 214 and 216***

Claim 211 depends directly from claim 208. Claim 211 recites that a primer used in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding site (page 10, lines 24-29; original claim 23).

Claim 214 depends indirectly from claim 208 via intermediate claims 212 and 213. Claim 214 recites that the label on the probes used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

Claim 216 depends indirectly from claim 190 via intermediate claims 212 and 215. Claim 198 recites that the label on the primers used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

### ***Independent Claim 217***

The subject matter of claim 217 embodies a process for the amplification of a target viral RNA and a reference RNA in a sample (page 2, lines 2-13; page 4, lines 1-9). The process comprises (i) combining a known quantity of a reference RNA sequence with the sample (page 7, lines 1-6 and 15-19), (ii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction (page 2, lines 7-8; page 8, line 26 – page 9, line 5; page 12, line 30-32) and then to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19) and (iii) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

***Dependent Claims 220, 223 and 225***

Claim 220 depends directly from claim 217. Claim 193 recites that a primer used in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding site (page 10, lines 24-29; original claim 23).

Claim 223 depends indirectly from claim 217 via intermediate claims 221 and 222. Claim 223 recites that the label on the probes used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

Claim 225 depends indirectly from claim 217 via intermediate claims 221 and 224. Claim 225 recites that the label on the primers used to measure the amount of amplified products is an isotope or a fluorophore (page 7, lines 20-22).

***Independent Claim 249***

The subject matter of claim 249 embodies a process for the quantitation of a target viral RNA in a sample (page 2, lines 13-15; page 7, lines 1-2 and 9-10). The process comprises (i) selecting a sequence present in the target viral RNA (page 4, line 22 – page 5, line 9; page 5, lines 10-25), (ii) adding a known quantity of a reference RNA sequence to the sample (page 7, lines 1-6 and 15-19), (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19), (iv) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19) and (v) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence (page 7, lines 2-8).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same

oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

### ***Independent Claim 250***

The subject matter of claim 250 embodies a process for the quantitation of a target viral RNA in a sample (page 2, lines 13-15; page 7, lines 1-2 and 9-10). The process comprises (i) selecting a sequence present in the target viral RNA (page 4, line 22 – page 5, line 9; page 5, lines 10-25), (ii) adding a known quantity of a reference RNA sequence to the sample (page 7, lines 1-6 and 15-19), (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction (page 2, lines 7-8; page 8, line 26 – page 9, line 5; page 12, line 30-32) and then to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19), (iv) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19) and (v) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence (page 7, lines 2-8).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

***Independent Claim 251***

The subject matter of claim 251 embodies a process for the quantitation of a target viral RNA sequence in a sample (page 2, lines 13-15; page 7, lines 1-2 and 9-10). The process comprises (i) combining a known quantity of a reference RNA sequence with the sample (page 7, lines 1-6 and 15-19), (ii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence to polymerase chain reaction amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19), (iii) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19) and (iv) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence (page 7, lines 2-8).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

***Independent Claim 252***

The subject matter of claim 252 embodies a process for the quantitation of a target viral RNA sequence in a sample (page 2, lines 13-15; page 7, lines 1-2 and 9-10). The process comprises (i) combining a known quantity of a reference RNA sequence with the sample (page 7, lines 1-6 and 15-19), (ii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction (page 2, lines 7-8; page 8, line 26 – page 9, line 5; page 12, line 30-32) and then to polymerase chain reaction

amplification under conditions to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence (page 4, lines 1-9; page 7, lines 1-6 and 15-19), (iii) measuring the amounts of amplified selected target viral RNA sequence and the amplified reference RNA sequence (page 7, lines 1-6 and 15-19) and (iv) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence (page 7, lines 2-8).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

### ***Independent Claim 253***

The subject matter of claim 253 embodies an amplification reaction mixture for the quantitation of a target viral RNA sequence in a biological sample (page 8, line 21 – page 9, line 7). The reaction mixture comprises (i) a target viral RNA sequence (page 4, line 22 – page 5, line 9; page 5, lines 10-25), (ii) a known quantity of a reference RNA sequence (page 7, lines 1-6 and 15-19) and (iii) an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified (page 4, lines 5-8 and 17-21).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified

selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

#### ***Independent Claim 254***

The subject matter of claim 254 embodies a reverse transcription mixture for reverse transcribing a target viral RNA sequence suspected of being present in a biological sample (page 8, line 21 – page 9, line 7). The reaction mixture comprises (i) a target viral RNA sequence (page 4, line 22 – page 5, line 9; page 5, lines 10-25), (ii) a known quantity of a reference RNA sequence (page 7, lines 1-6 and 15-19) and (iii) an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified (page 4, lines 5-8 and 17-21).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

The oligonucleotide primer pair is for initiating cDNA synthesis to provide a target viral cDNA and a reference sequence cDNA (page 2, lines 7-8 and 27-30; page 8, line 21 – page 9, line 7). Following reverse transcription, the target viral and reference sequence cDNAs can serve as templates for amplification (page 2, lines 7-8; page 2, line 27 – page 3, line 11; page 9, lines 2-7) for providing amplified reference RNA sequence and amplified target viral RNA sequence (page 3, lines 5-11; page 9, lines 24-27; page 12, lines 30-32).

#### ***Independent Claim 255***

The subject matter of claim 255 embodies a kit for the quantitation of a target viral RNA sequence in a biological sample (page 13, lines 1-11). The kit comprises individual containers

(page 11, lines 4-9) which provide (i) a known quantity of a reference RNA sequence (page 7, lines 1-6 and 15-19; page 11, lines 4-9) and (ii) an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified (page 4, lines 5-8 and 17-21).

The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15) and a sequence not present in the selected target viral RNA sequence (page 6, lines 19-25; page 7, lines 10-15). The reference RNA sequence and the selected target viral RNA sequence can be amplified by the same oligonucleotides (page 4, lines 17-21; page 6, lines 15-18) or different oligonucleotides (page 4, lines 5-8). Following amplification, the amplified reference RNA sequence and the amplified selected target viral RNA sequence are distinguishable by size (page 6, lines 26-29; page 7, lines 12-15) or by probes (page 7, lines 25-27).

#### **VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL**

- A. Whether claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224, 226-248 and 249-255 are unpatentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).
- B. Whether claims 193, 196, 198, 202, 205, 207, 211, 214, 216,<sup>4</sup> 220, 223 and 225 are unpatentable under 35 U.S.C. § 135(b) over Wang et al. in view of Mullis et al. (US 4,683,195).

#### **VII. ARGUMENT**

- A. **Claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224, 242-245 and 249-255 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).**

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<sup>4</sup> Although claim 216 was indicated in the Summary portion of the Office Action dated December 23, 2008 as being rejected, claim 216 was not specifically listed in either of the two rejections set forth in this Office Action. Since claim 216 is similar to claims 198, 207 and 225 which are specifically listed in this rejection over Wang et al. in

The Examiner rejected claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224, 226-248 and 249-255 as being unpatentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727). (Office Action, pages 3-4, ¶ 5).<sup>5</sup> The Examiner contends that the claims require the use of a shared primer pair that is taught by Wang et al. and thus are not patentable. Appellants respectfully submit that the Examiner is in error in this rejection.

**1. Appellants are entitled to priority of U.S. Serial No. 07/148,959 filed January 27, 1988.**

As discussed in further detail below, it is Appellants' position that the claimed subject matter is not barred by 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727). Since the claims are not barred by 35 U.S.C. § 135(b), Wang et al. is then potentially prior art under 35 U.S.C. § 102(e). The present application is a continuation-in-part of application Serial No. 07/148,959 (hereinafter "the '959 application") filed January 27, 1988. The rejections made by the Examiner are predicated, in part, on his contention that the instant application is not entitled to priority of the '959 application. Appellants submit that the Examiner is in error in this contention.

The Examiner contends in the Office Action (p. 2, ¶ 3) that the limitation "same oligonucleotides" does not have support in the earlier filed application Serial No. 07/148,959 ('959 application) and thus, the instant application does not receive priority to January 27, 1988. In arriving at this conclusion, the Examiner contends that the reference RNA disclosed at page 3, lines 23-26 of the '959 application must refer to a beta actin gene in view of the disclosure at page 3, lines 12-22 of the '959 application. Specifically, the Examiner states

After reviewing the patent application '959 and the cited paragraph, and the preceding paragraphs on page 3 of the specification of patent application '959, it is noted that the reference RNA in the cited paragraph is referring to a beta actin gene, and not to the reference maxigene as asserted by the Applicants.

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view of Mullis et al., Applicants believe that the Examiner should have included claim 216 in this rejection and include it as such in their arguments.

<sup>5</sup> Unless noted otherwise, all citations to the Office Action are to the Office Action dated December 23, 2008.

(Office Action, page 2, ¶ 3). The Examiner also contends that Example III of the '959 application discloses the use of a separate primer for maxigene amplification. Specifically, the Examiner

further notes on page 7 of the specification of patent application '959 use of a separate primer for maxigene amplification (see example III of the patent application '959 on page 7).

(Office Action, page 2, ¶ 3). Appellants respectfully submit that the Examiner is in error in his analysis and conclusion concerning the teachings of the '959 application and furthermore has not considered the disclosure of the '959 application as a whole.

Initially, it appears that the Examiner may have been confused by the use of the terms "fourth primer," "maxigene primer" and "maxigene" in the '959 application. Although the '959 application does mix up the use of the terms "primer" and "reference RNA" with respect to the "maxigene," Appellants submit that the remainder of the '959 application is clear that the specified "fourth primer," "maxigene primer" and "maxigene" are all a reference RNA sequence. Appellants believe that the draftsman of the '959 application could have eliminated any confusion in use of the terms "fourth primer," "maxigene primer" and "maxigene" if he had been consistent in the use of the terms "primer," "maxigene" and "reference RNA sequence." Nevertheless, Appellants submit that it is clear from the '959 application that each of the specified "fourth primer," "maxigene primer" and "maxigene" terms used in the '959 application is a reference RNA sequence for a target viral RNA and is not the beta actin gene as asserted by the Examiner.

The '959 application is directed to the simultaneous amplification of a viral RNA present in a sample with at least one other RNA sequence. ('959 application, page 1, lines 20-24 and page 2, lines 2-4). The amplification of this other RNA sequence can provide a positive control. ('959 application, page 1, lines 24-25 and page 2, lines 2). Three examples of target viral RNA are described on page 2, lines 6-32 of the '959 application, namely one target from HIV-1 and two targets from HCMV. Primers and probes for amplifying and detecting each of these target sequences are described on page 2 of the '959 application. The "first primer pair" is used to amplify the viral RNA. ('959 application, page 2, lines 6-7).

The '959 application discloses that T-4 lymphocytes are primarily affected by HIV-1 and that T-4 lymphocytes express the T-cell receptor. ('959 application, page 2, lines 33-35). A "second primer pair" is used to amplify a sequence which is unique to the T-cell receptor." ('959 application, page 3, lines 1-3). Primers and a probe for amplifying and detecting the T-cell receptor are described at page 3, lines 7-11 of the '959 application. The specification then describes a "third primer pair" which is used to amplify an RNA sequence present, in all cells of a peripheral blood sample, preferably a beta actin sequence. ('959 application, page 3, lines 12-16). Primers and a probe for amplifying and detecting a beta actin sequence are described at page 3, lines 18-22 of the '959 application.

Example I of the '959 application describes the simultaneous amplification of an HIV-1 sequence and a T-cell receptor sequence using the HIV-1 primers disclosed on page 2 of the '959 application and the T-cell receptor primers disclosed on page 3 of the '959 application. The amplified HIV-1 sequence is detected using the HIV-1 probe disclosed on page 2 of the '959 application. The amplified T-cell receptor sequence is detected using the T-cell receptor probe disclosed on page 3 of the '959 application.

Example II of the '959 application describes the amplification of an HIV-1 sequence, a T-cell receptor sequence and a beta actin sequence. The HIV-1 sequence and T-cell receptor sequence are amplified and detected as described in Example I. The beta actin sequence is amplified using the beta actin primers disclosed on page 3 of the '959 application, and the amplified beta actin sequence is detected using the beta actin probe disclosed on page 3 of the '959 application.

It is clear from the description of the '959 application that the T-cell receptor and the beta actin gene are used as reference RNA sequences. It was well known to the skilled artisan at the filing date of the '959 application that HIV-1 and HCMV are viruses and that the sequences of HIV-1 or HCMV amplified in accordance with the '959 application are viral RNA sequences. It was also well known to the skilled artisan that the T-cell receptor and beta actin are neither viruses nor viral RNA genes.

According to the '959 application,

A fourth primer ... is provided by a reference RNA sequence which can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples."

For HIV-1 such a reference RNA may be a "maxigene" formed by a multi-base pair insert into a unique site for example the unique KpnI site of the 3' ORF region. A preferred reference RNA includes a 22 base pair insert into the KpnI site of the HIV-1 3' ORF region of the pGEM92 clone described by Murakawa.

('959 application, page 3, lines 23-31). Appellants submit that it is clear to the skilled artisan reading this passage that the "fourth primer" is a "reference RNA sequence" and that such a "reference RNA sequence" may be a "maxigene." The pGEM92 clone is a 1.1 kb BamHI restriction fragment of HIV-1 inserted into the pGEM2 vector. ('959 application, page 4, lines 3-10). The '959 application also discloses that the amplification

product of this sequence is 22 bases longer than the authentic HIV-sequence but still hybridizes to the 25 mer probe HIVC. It is therefore distinguishable by size from the authentic viral product.

('959 application, page 4, lines 11-14). The '959 application further discloses that

The "maxigene" provides an internal control and an additional aid to quantitation.

('959 application, page 5, lines 1-2).

Appellants submit that it is clear to the skilled artisan reading the disclosure of the '959 application that three potential reference sequences can be used and simultaneously amplified with the target viral RNA sequence. The first reference sequence is a T-cell receptor sequence. The second reference sequence is a beta actin sequence. The third reference sequence is "fourth primer" or "maxigene." The plain language of the '959 application states that the "fourth primer" is a **reference RNA that can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples**. On the basis of the disclosure at page 3, lines 23-31 of the '959 application, Appellants submit that the skilled artisan understands that the "fourth primer" is a reference RNA that comprises a viral RNA sequence. The '959 application then states that "[F]or HIV-1 such a reference RNA may be a 'maxigene.'" Thus, Appellants submit that the skilled artisan reading the '959 application understands that the "fourth primer" is a "reference RNA" which is the "maxigene." In view of the specific language found on page 3 of

the '959 application, Appellants submit that the skilled artisan understands that the "fourth primer" can be amplified by the same oligonucleotides as a target viral RNA sequence. Because the same oligonucleotides can be used to amplify both the "fourth primer," i.e., "reference RNA," and the viral RNA sequence, it is apparent to the skilled artisan that the "fourth primer," i.e., "reference RNA," is a viral sequence. Since beta actin is not a viral sequence, Appellants submit that the "fourth primer," i.e., "reference RNA," cannot be beta actin, and the skilled artisan reading the '959 application would know that the "fourth primer" is a "reference RNA" which is a viral sequence and not a beta actin sequence. Consequently, Appellants respectfully submit that the Examiner's contention that the reference RNA disclosed at lines 23-26 on page 3 of the '959 application refers to a beta actin sequence is incorrect.

In addition, page 3 of the '959 application describes one example of a reference RNA for HIV-1. This reference RNA is a maxigene, which is formed by a multi-base insert into the 3' ORF of HIV-1. Furthermore, page 3, line 27 of the '959 application states "[F]or HIV-1 **such** a reference RNA may be a maxigene." (emphasis added) It is apparent to the skilled artisan reading the '959 application that the term "**such**" refers to the reference RNA in the immediately preceding paragraph (i.e., page 3, line 25). This reference RNA is amplified by the same oligonucleotides as the viral RNA (page 3, lines 25-26). Thus, Appellants submit that it is apparent to the skilled artisan that the maxigene is a reference RNA which includes a viral RNA sequence and that the maxigene cannot refer to a beta actin sequence. Consequently, Appellants respectfully submit that the Examiner's contention that the reference RNA disclosed at lines 23-26 on page 3 of the '959 application refers to a beta actin sequence and not a maxigene as a reference RNA sequence is not correct.

Furthermore, Appellants submit that Example III of the '959 application does not describe the use of a separate primer for maxigene amplification as asserted by the Examiner. Example III describes the simultaneous amplification of the HIV-1 viral sequence, the T-cell receptor sequence and the "maxigene" sequence. That is, Example III states that Example I is repeated with the exception that the "maxigene primer" is included in the reaction mixture. As detailed above, Appellants submit that the "maxigene primer" is the "fourth primer," i.e., the "maxigene." No other primer is described in the '959 application. As described above, Example

I discloses the simultaneous amplification of an HIV-1 sequence and a T-cell receptor sequence. Example I does not include the amplification of the beta actin gene. Thus, the nucleic acids amplified in Example III include the HIV-1 sequence, the T-cell receptor sequence and the maxigene. The primers included in Example I are the primers for the HIV-1 sequence and the primers for the T-cell receptor sequence. Example I and thus Example III do not include primers for the beta actin gene. Appellants submit that it is apparent to the skilled artisan that the only primers that are available in Example III for amplifying the nucleic acids present in the reaction mixture are those that are utilized in Example I, i.e., the only primers in Example III are the HIV-1 primers and the T-cell receptor primers. Since the maxigene, as disclosed on page 3 of the '959 application, comprises a multi-base pair insert into the 3' ORF of HIV-1, Appellants submit that it is apparent to the skilled artisan that the T-cell receptor primers will not amplify the maxigene sequence. Thus, Appellants submit that the skilled artisan knows from Example III that the HIV primers amplify both the HIV-1 viral sequence and the maxigene. Consequently, Appellants submit that Example III of the '959 application discloses that the same oligonucleotides are used to amplify both the target viral RNA sequence and the reference RNA sequence.

In the Advisory Action,<sup>6</sup> the Examiner states that

example III of the '959 application discloses that the experiment I is repeated with the addition of a primer for maxigene, which clearly indicate that the experiment III utilizes maxigene primer in addition to the primer pair that amplify the authentic virus RNA and does not disclose the use of the same primers to amplify both the target and the reference RNA (HIVA, HIVB, T-cell receptor A and B and maxigene target as a reference RNA) simultaneously.

(Advisory Action, page 2). However, Example III on page 7 of the '959 application states that

Example I is repeated with the exception that the maxigene primer is included in the reaction mixture.

Example III does not state that a primer for the maxigene is added to the reaction mixture of Example I as asserted by the Examiner. As discussed above, it is clear to the skilled artisan reading the '959 application that the maxigene primer referred to in Example III of the '959

application is the reference RNA referred to on page 3 of the '959 application as the "fourth primer" and as the "maxigene." Furthermore, as the skilled artisan was aware at the filing date of the '959 application, two primers are used in a polymerase chain reaction amplification. There is no disclosure in Example III that two primers are added to amplify the maxigene. Thus, Appellants submit that the skilled artisan reading the '959 application would know that the same HIVA and HIVB primers are used to amplify both the HIV sequence and the maxigene.

In view of the above analysis of the '959 application, Appellants submit that the maxigene is a reference RNA that can be amplified and detected using the same oligonucleotides as the target viral RNA. Thus, Appellants submit that the present application is entitled to priority of the '959 application filed on January 27, 1988.

**2. Claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224 and 242-245 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).**

Claims 190-192, 194, 195, 197, 199-201, 203, 204, 205, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224 and 242-245 are directed to **a process for the amplification of target viral RNA and a reference RNA in a sample**. These claims are **not** directed to the quantitation of a target viral RNA in a sample. Independent claims 190, 199, 208 and 217 include the steps of (a) adding a known quantity of a reference RNA sequence to a sample, (b) simultaneously subjecting the target viral RNA sequence and the reference RNA sequence to PCR amplification and (c) measuring the amounts of amplified selected viral RNA sequence and amplified reference sequence. Independent claims 190 and 199 further include an initial step of selecting a sequence present in the target viral RNA prior to adding the reference RNA sequence. Independent claims 199 and 217 further include a first reverse transcription reaction in step (b) followed by amplification. The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence. The reference RNA sequence and the target viral RNA sequence can be amplified by

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<sup>6</sup> Unless noted otherwise, all citations to the Advisory Action are to the Advisory Action dated June 16, 2009.

the same or different oligonucleotides. Following amplification, the amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes. In step (b), the target viral RNA sequence if present in the sample and the reference RNA sequence are simultaneously amplified. It is evident from reading independent claims 190, 199, 208 and 217 that there is no step for calculating the amount of target viral RNA present in the sample before amplification. That is, independent claims 190, 199, 208 and 217 **do not** include a step for

calculating from the amplified target and standard segments produced in step (d) the amount of said target nucleic acid segment present in the sample before amplification.

This calculation step is required by the claims of Wang et al. See, for example, claim 1 of Wang et al. which requires in step (f) the calculation of the amount of the target nucleic acid present in the sample before amplification using the precise language set forth above. Appellants also note that the preamble of claim 1 of Wang et al. specifies that the method is for quantifying a target nucleic acid in a sample. In order to quantify the target nucleic acid in a sample, it is necessary to calculate the amount of the target nucleic acid in the sample before amplification as set forth in step (f) of claim 1 of Wang et al. The calculation step in Wang et al. is performed after amplification. There is no such calculation step in any of independent claims 190, 199, 208 and 217.

As amply illustrated above, the subject matter of independent claims 190, 199, 208 and 217 **do not include a quantification step**, i.e., a step of calculating the amount of target nucleic acid initially present in the sample. Because independent claims 190, 199, 208 and 217 of the present application do not include a step of calculating the amount of target nucleic acid in the sample before amplification, i.e., do not include a step for quantifying a target nucleic acid in a sample, Appellants submit that the claimed subject matter is neither the same nor substantially the same as the claimed subject matter of Wang et al. Thus, Appellants respectfully submit that Wang et al. does not anticipate independent claims 190, 199, 208 and 217 under 35 U.S.C. § 135(b).

In the Advisory Action, the Examiner contends that

Applicants' argue that Wang et al. disclose calculating the amount of target nucleic acid initially present in the sample **before amplification step**, which is not required by the instant claims 190-225, and 242-255.

(Advisory Action, page 2, emphasis added). However, Appellants have not made this argument. That is, Appellants have not argued that the amount of target viral RNA is determined before the amplification step is performed as asserted by the Examiner. Instead, Appellants have argued that independent claims 190, 199, 208 and 217 do not include a step of calculating the amount of target viral RNA present in the original sample, i.e., the amount of target viral RNA present in the sample before amplification, as required by Wang et al. That is, Appellants have argued that the claimed subject matter does not include a calculation step as required by step (f) of Wang et al. Although this calculation is done after the amplification step, the calculation determines the amount of target viral RNA present in the sample before amplification. Thus, Appellants submit that independent claims 190, 199, 208 and 217 do not claim the same or substantially the same subject matter as Wang et al. because there is no calculation step, i.e., there is no step for calculating the amount of target viral RNA present in the sample on the basis of the amounts of the amplified products.

Claims 191, 192, 194, 195, 197 and 242 depend directly or indirectly from claim 190 and thus incorporate all of the limitations of claim 190. None of these claims include a calculation step.

Claims 200, 201, 203, 204, 206 and 243 depend directly or indirectly from claim 199 and thus incorporate all of the limitations of claim 199. None of these claims include a calculation step.

Claims 209, 210, 212, 213, 215 and 244 depend directly or indirectly from claim 208 and thus incorporate all of the limitations of claim 208. None of these claims include a calculation step.

Claims 218, 219, 221, 222, 224 and 245 depend directly or indirectly from claim 217 and thus incorporate all of the limitations of claim 217. None of these claims include a calculation step.

Accordingly, Appellants submit that claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224 and 242-245 are not anticipated by Wang et al. under 35 U.S.C. § 135(b). Reversal of this rejection is respectfully requested.

In addition, Appellants note that Wang et al. is not prior art under other sections of Title 35 U.S.C., including 35 U.S.C. § 102(e). Specifically, Appellants note that Wang et al. was filed on September 28, 1989 claiming priority to an application filed on August 21, 1989. Thus, the earliest effective filing date for Wang et al. is August 21, 1989. As described above, Appellants are entitled to priority to U.S. Serial No. 07/148,959 filed on January 27, 1988. This priority date antedates the earliest effective filing date of Wang et al. Furthermore, Appellants submitted a Declaration Under 37 CFR 1.131(a)<sup>7</sup> (hereinafter "131 Declaration") on December 15, 2005. This 131 Declaration swears behind the earliest effective filing date of Wang et al. The evidence submitted with the Rule 131 Declaration to establish an earlier date of invention is a draft of the present application. In view of this 131 Declaration, Appellants submit that Wang et al. is not prior art under other sections of Title 35 U.S.C. (i.e., any section other than Section 135(b)).

In the Advisory Action, the Examiner contends that Wang et al. is prior art because it teaches use of the same primers to amplify target and reference RNA and because use of the same primers in the present application is not supported in the priority application (i.e., the '959 application). As demonstrated above, the present application is entitled to priority to the '959 application. Furthermore, the Examiner did not consider the 131 Declaration which removes Wang et al. as prior art under other sections of Title 35 U.S.C., including 35 U.S.C. § 102(e). Thus, Appellants submit that Wang et al. is not prior art to the claimed subject matter with respect to other sections of Title 35 U.S.C. for at least the reason that it has been removed as prior art by the 131 Declaration. Therefore, Appellants respectfully submit that claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224 and 242-245 are patentable over Wang et al. with respect to other sections of Title 35 U.S.C., including 35 U.S.C. § 102(e).

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<sup>7</sup> A copy of the Declaration Under 37 CFR 1.131(a) filed on December 15, 2005 is included in Appendix IX.

Accordingly, Appellants submit that claims 190-192, 194, 195, 197, 199-201, 203, 204, 206, 208-210, 212, 213, 215, 217-219, 221, 222, 224 and 242-245 are not anticipated by Wang et al. under 35 U.S.C. § 135(b) and are otherwise patentable over Wang et al. Reversal of this rejection is respectfully requested.

**3. Claims 249-252 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).**

Claims 249-252 are directed to **a process for the quantitation of target viral RNA in a sample**. In accordance with this process, (i) a known quantity of a reference RNA sequence is added to a sample containing a viral RNA sequence, (ii) the target viral RNA sequence and the known quantity of the reference RNA sequence are simultaneously amplified, (iii) the amounts of amplified products are measured and (iv) the relative amount of the target viral RNA present in the sample before amplification is determined from the amounts of the amplified target viral RNA sequence and the amplified reference RNA sequence. Independent claims 249 and 250 further include an initial step of selecting a sequence present in the target viral RNA prior to adding the reference RNA sequence. Independent claims 250 and 250 further include a first reverse transcription reaction in step (ii) followed by amplification. The reference RNA sequence can be used as an internal standard. The reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA sequence. The reference RNA sequence and the target RNA sequence can be amplified by the same or different oligonucleotides. The amplified target viral RNA sequence and the amplified reference RNA sequence can be distinguished by size or by probes.

Although these claims relate to a process for the quantitation of a target viral RNA in a sample, Appellants submit that they are not claiming the same or substantially the same subject matter as Wang et al. because (1) the claimed subject matter does not require the use of a shared primer pair and (2) the claimed subject matter includes the step of determining the relative amount of target viral RNA before amplification. More specifically and as detailed below, Appellants submit that the claimed subject matter **does not require** the use of a shared primer pair, as was held by the Board in Interference No. 105,055. Appellants further submit that the

determination of a relative amount of target viral RNA distinguished Wang et al. from Appellants proposed claim containing this limitation as was held by the Board in the interference. Because the claimed subject matter **does not require** the use of a shared primer pair and specifies determination of the relative amount of target viral RNA, Appellants submit that Wang et al. is not prior art under 35 U.S.C. § 135(b).

With respect to claims 249-252, Appellants submit that these claims are patentable under 35 U.S.C. § 135(b) over Wang et al. in view of the Board's decisions in Interference No. 105,055. First, Appellants submit that the Board's decision on Wang preliminary motion 1 (Paper 36 dated 5 November 2003 and titled Memorandum Opinion and Order<sup>8</sup>) (hereinafter "Memorandum Opinion and Order") held that that Appellants are not claiming the same subject matter as claimed in the Wang et al. In its decision on Wang preliminary motion 1 the Board stated

the dispositive question is whether "a reference RNA which **can be amplified and detected by the same oligonucleotides** as used for authentic virus RNA samples" necessarily **requires** or results in the use of a shared primer pair.

(Memorandum Opinion and Order, page 20; emphasis added). According to original claim 19, one of the reference sequences could be a "sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence." The specification describes a maxigene for the HIV-1 target which is multi-base insert into a unique site in the target sequence. The unique site for the exemplified maxigene in the specification was between the primer binding sites of the target sequence, and thus the same primers would be used to amplify the target sequence and the reference sequence. The Board concluded that there was no disclosure in the specification that the preselected site set forth in original claim 19 should be chosen to avoid disrupting primer binding sites and noted that the claims could not be limited to a preferred embodiment. (Memorandum Opinion and Order, pages 21-22). Thus, the Board concluded that although the Murakawa et al. earlier claims "encompass use of a shared primer pair, they **do not require** or necessarily result in use of a

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<sup>8</sup> Entered as Paper No. 49 in the present application which is included in Appendix X.

shared primer pair.” (Memorandum Opinion and Order, page 22; emphasis added). In this regard, the Board stated “[I]t is possible to have a maxigene control sequence which can be amplified by different primers and detected by the same oligonucleotides used for the target sequence.” (Memorandum Opinion and Order, page 22). The Board concluded that “binding to a shared primer pair is **neither excluded, required nor a necessary result**” in any of the Murakawa et al. earlier claims. (Memorandum Opinion and Order, page 22; emphasis added). Thus, the Board concluded that none of the earlier Murakawa claims were directed to the same or substantially the same invention as claimed in Wang et al. (Memorandum Opinion and Order, pages 22-23). These earlier claims included claims that, when read in light of the specification, include a reference RNA that can be amplified and detected by the same oligonucleotides as the target RNA.

Thus, the Board specifically held that the language “can be amplified and detected by the same oligonucleotides,” **did not require** or necessarily result in the use of a shared primer pair. Thus, Appellants submit that the Board has held that this language **does not require** the use of a shared primer pair. It is clear that the language of the claims “can be amplified by the same or different oligonucleotides” does not require use of a shared primer pair because different primers can be used by the plain language of the claims. If different primers can be used to amplify both the target viral RNA sequence and the reference RNA sequence, then it is clear that the use of a shared primer pair is not required, and it is clear that the method does not necessarily result in the use of a shared primer pair. Because the claims do not require the same primer pair, they are not barred by 35 U.S.C. § 135(b).

In addition, the term “can” in grammatical usage is used to merely express an ability. Ability means that it may happen, but there is no requirement that it must happen. Thus, Appellants submit that the term “can” as used in the claimed subject matter does not require or necessarily result in the use of a shared primer pair. Because the term “can” does not require the same primer pair, Appellants submit that the claims are not barred by 35 U.S.C. § 135(b).

Second, Appellants note that the final step in claims 249-252 recites “determining the **relative** amount of the target viral RNA sequence present in the sample before amplification.” This step was one of the steps that the Board noted was different than the corresponding step in

Wang et al. in concluding that Wang et al. claim 1 was not obvious over Murakawa's proposed claim 50 in combination with the prior art in the Board's decision on Murakawa preliminary motion 1 (Paper 47 dated April 5, 2004 and titled Decision on Preliminary Motion<sup>9</sup>) (hereinafter "Decision on Preliminary Motion"). Specifically, the Board stated

First, although the preamble of proposed claim 50 recites a "process for quantitation of a target viral RNA sequence, "proposed claim 50 does *not* contain the step of calculating the [absolute] amount of target RNA in the sample that is found in claim 1 of the Wang '727 patent" (Wang's opposition, Paper 44, p. 22). Instead, proposed claim 50 recites "determining the **relative quantification** of the target sequence" (step (v), emphasis added [in original]).

(Decision on Preliminary Motion, page 25; emphasis added). If the determination of the "relative amount" as required by claims 249-252 is inherently the same as calculating the absolute amount of target RNA as required by claim 1 of Wang et al. '727, then the Board could not have reached this conclusion. That is, the Board did not consider that determining the relative amount was inherent because it would then have had to conclude that, at least on the basis of this step, the method of Wang et al. would have been obvious over the proposed Murakawa et al. claim. Because the Board did not so find, Appellants submit that the Examiner's contention concerning inherency with respect to step (v) of claim 249, i.e., determining the relative amount of target viral RNA, is not correct and contrary to the holding by the Board. For the above reasons, Appellants submit that claims 249-252 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).

Furthermore, as described above in Section VII.A.2, Wang et al. is not prior art under other section of Title 35 U.S.C. (i.e., any section other than Section 135(b)), including 35 U.S.C. § 102(e), with respect to claims 249-252 because (i) the present application has priority to U.S. Serial No. 07/148,959 filed on January 27, 1988 which antedates the earliest filing date for Wang et al. and/or (ii) Wang et al. has been removed as prior art by the Declaration Under 37 CFR 1.131(a) filed on December 15, 2005 that establishes an earlier date of invention for the present

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<sup>9</sup> Entered as Paper No. 50 in the present application which is included in Appendix X.

application. Therefore, Appellants submit that claims 249-252 are patentable over Wang et al. with respect to other sections of Title 35 U.S.C.

Accordingly, Appellants submit that claims 249-252 are not anticipated by Wang et al. under 35 U.S.C. § 135(b) and are otherwise patentable over Wang et al. Reversal of this rejection is respectfully requested.

**4. Claims 253-255 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).**

Claims 253-255 are directed to reaction mixtures and kits as described below. These claims are not directed to a method for the quantitation of a target viral RNA and thus do not include a method step for determining the relative amount of target viral RNA as set forth in claims 249-252. Thus, Appellants submit that claims 249-252 are separately patentable from claims 253-255, because the determination step in claims 249-252 provides additional basis for the patentability of these claims as detailed above.

Claim 253 is directed to an amplification reaction mixture for quantitation of target viral RNA. The amplification reaction mixture comprises a target viral RNA sequence, a known quantity of a reference RNA sequence and an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified.

Claim 254 is directed to a reverse transcription mixture for reverse transcribing target viral RNA. The reverse transcription reaction mixture comprises a target viral RNA sequence, a known quantity of a reference RNA sequence and an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified. The oligonucleotide primer pair is for initiating cDNA synthesis to provide a target viral cDNA and a reference sequence cDNA, each of which can serve as templates for amplification.

Claim 255 is directed to a kit for quantitation of target viral RNA. The kit comprises a known quantity of a reference RNA sequence and an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified.

The reference RNA sequence in each of claims 253-255 comprises a sequence present in the target viral RNA sequence and a sequence that is not present in the target viral RNA

sequence. The reference RNA sequence and the target RNA sequence can be amplified by the same or different oligonucleotides. The amplified target viral RNA sequence and the amplified reference RNA sequence can be distinguished by size or by probes.

With respect to claims 253-255, Appellants submit that these claims are patentable under 35 U.S.C. § 135(b) over Wang et al. in view of the Board's decisions in Interference No. 105,055. Specifically, Appellants submit that the Board's Memorandum Opinion and Order decision held that that Appellants are not claiming the same subject matter as claimed in the Wang et al. In its decision on Wang preliminary motion 1 the Board stated

the dispositive question is whether “a reference RNA which **can be amplified and detected by the same oligonucleotides** as used for authentic virus RNA samples” necessarily **requires** or results in the use of a shared primer pair.

(Memorandum Opinion and Order, page 20; emphasis added). According to original claim 19, one of the reference sequences could be a “sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence.” The specification describes a maxigene for the HIV-1 target which is multi-base insert into a unique site in the target sequence. The unique site for the exemplified maxigene in the specification was between the primer binding sites of the target sequence, and thus the same primers would be used to amplify the target sequence and the reference sequence. The Board concluded that there was no disclosure in the specification that the preselected site set forth in original claim 19 should be chosen to avoid disrupting primer binding sites and noted that the claims could not be limited to a preferred embodiment. (Memorandum Opinion and Order, pages 21-22). Thus, the Board concluded that although the Murakawa et al. earlier claims “encompass use of a shared primer pair, they **do not require** or necessarily result in use of a shared primer pair.” (Memorandum Opinion and Order, page 22; emphasis added). In this regard, the Board stated “[I]t is possible to have a maxigene control sequence which can be amplified by different primers and detected by the same oligonucleotides used for the target sequence.” (Memorandum Opinion and Order, page 22). The Board concluded that “binding to a shared primer pair is **neither excluded, required nor a necessary result**” in any of the Murakawa et al. earlier claims. (Memorandum Opinion and Order, page 22; emphasis added).

Thus, the Board concluded that none of the earlier Murakawa claims were directed to the same or substantially the same invention as claimed in Wang et al. (Memorandum Opinion and Order, pages 22-23). These earlier claims included claims that, when read in light of the specification, include a reference RNA that can be amplified and detected by the same oligonucleotides as the target RNA.

Thus, the Board specifically held that the language “can be amplified and detected by the same oligonucleotides,” **did not require** or necessarily result in the use of a shared primer pair. Thus, Appellants submit that the Board has held that this language **does not require** the use of a shared primer pair. It is clear that the language of the claims “can be amplified by the same or different oligonucleotides” does not require use of a shared primer pair because different primers can be used by the plain language of the claims. If different primers can be used to amplify both the target viral RNA sequence and the reference RNA sequence, then it is clear that the use of a shared primer pair is not required, and it is clear that the method does not necessarily result in the use of a shared primer pair. Because the claims do not require the same primer pair, they are not barred by 35 U.S.C. § 135(b).

In addition, the term “can” in grammatical usage is used to merely express an ability. Ability means that it may happen, but there is no requirement that it must happen. Thus, Appellants submit that the term “can” as used in the claimed subject matter does not require or necessarily result in the use of a shared primer pair. Because the term “can” does not require the same primer pair, Appellants submit that the claims are not barred by 35 U.S.C. § 135(b). For the above reasons, Appellants submit that claims 253-255 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727).

Furthermore, as described above in Section VII.A.2, Wang et al. is not prior art under other section of Title 35 U.S.C. (i.e., any section other than Section 135(b)), including 35 U.S.C. § 102(e), with respect to claims 253-255 because (i) the present application has priority to U.S. Serial No. 07/148,959 filed on January 27, 1988 which antedates the earliest filing date for Wang et al. and/or (ii) Wang et al. has been removed as prior art by the Declaration Under 37 CFR 1.131(a) filed on December 15, 2005 that establishes an earlier date of invention for the present

application. Therefore, Appellants submit that claims 253-255 are patentable over Wang et al. with respect to other sections of Title 35 U.S.C.

Accordingly, Appellants submit that claims 253-255 are not anticipated by Wang et al. under 35 U.S.C. § 135(b) and are otherwise patentable over Wang et al. Reversal of this rejection is respectfully requested.

**B. Claims 193, 196, 198, 202, 205, 207, 211, 214, 216, 220, 223 and 225 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727) in view of Mullis et al. (US 4,683,195).**

The Examiner rejected claims 193, 196, 198, 202, 205, 207, 211, 214, 216, 220, 223 and 225 as being unpatentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727) in view of Mullis et al. (US 4,683,195). (Office Action, page 4, ¶ 6). The Examiner contends that the claims require the use of a shared primer pair that is taught by Wang et al. and thus are not patentable over Wang et al. in view of Mullis et al. Appellants respectfully submit that the Examiner is in error in this rejection.

**1. Appellants are entitled to priority of U.S. Serial No. 07/148,959 filed January 27, 1988.**

As discussed above in Section VII.A.1, it is Appellants' position that the claimed subject matter is entitled to priority to application Serial No. 07/148,959 filed on January 27, 1988.

**2. Claims 193, 196, 198, 202, 205, 207, 211, 214, 216, 220, 223 and 225 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727) in view of Mullis et al. (US 4,683,195).**

Claims 193, 196, 198, 202, 205, 207, 211, 214, 216, 220, 223 and 225 are directed to a **process for the amplification of target viral RNA and a reference RNA in a sample**. Since these claims depend from independent claims 190, 199, 208 and 217, they include the steps of (a) adding a known quantity of a reference RNA sequence to a sample, (b) simultaneously subjecting the target viral RNA sequence and the reference RNA sequence to PCR amplification

and (c) measuring the amounts of amplified selected viral RNA sequence and amplified reference sequence. The reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence. The reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides. Following amplification, the amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes. In step (b), the target viral RNA sequence if present in the sample and the reference RNA sequence are simultaneously amplified.

Appellants submit that these claims are directed to a process for amplification of a target viral RNA and a reference RNA in a sample. As discussed above in Section VII.A.2, these claims do not include a quantitation step as required by the claims of Wang et al. The quantitation step of Wang et al. is set forth in step (f) of claim 1. There is **no** corresponding quantitation step in the claimed subject matter. Since the claims do not require a quantitation step as required by Wang et al., Appellants submit that the claimed subject matter is not the same or substantially the same as the claimed subject matter of Wang et al. for the reasons detailed above in Section VII.A.2. That is, the claimed subject matter **does not include a quantification step**, i.e., a step of calculating the amount of target nucleic acid initially present in the sample. Since the claimed subject matter of the present application is not the same or substantially the same as the Wang et al. claimed subject matter, Appellants submit that Wang et al. is not a bar to patentability under 35 U.S.C. § 135(b). Since Mullis et al. does not disclose or suggest the claimed subject matter and does not cure the deficiencies of Wang et al., Appellants submit that Mullis et al. in combination with Wang et al. does not render the claimed subject matter obvious. Thus, Appellants submit that claims 193, 196, 198, 202, 205, 207, 211, 214, 216, 220, 223 and 225 are patentable under 35 U.S.C. § 135(b) over Wang et al. (US 5,219,727) in view of Mullis et al.

Furthermore, as described above in Section VII.A.2, Wang et al. is not prior art under any other section of Title 35 U.S.C. (i.e., any section other than Section 135(b)) with respect to claims 190-225 and 242-245, and therefore claims 193, 196, 198, 202, 205, 207, 211, 214, 220,

223 and 225 are patentable over Wang et al. with respect to such sections of Title 35 U.S.C. in view of Mullis et al.

Accordingly, Appellants submit that claims 193, 196, 198, 202, 205, 207, 211, 214, 220, 223 and 225 are not rendered unpatentable under 35 U.S.C. § 135(b) over Wang et al. and Mullis et al., and are otherwise patentable over Wang et al. in view of Mullis et al. Reversal of this rejection is respectfully requested.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By           /Jeffrey L. Ihnen/          

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### **VIII. CLAIMS APPENDIX**

1-189: (Canceled).

190: A process for amplification of a target viral RNA and a reference RNA in a sample which comprises:

- (i) selecting a sequence present in the target viral RNA;
- (ii) adding a known quantity of a reference RNA sequence to the sample, wherein the reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence, wherein the reference RNA sequence and the selected target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified selected target viral RNA sequence are distinguishable by size or by probes;
- (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence; and
- (iv) measuring the amounts of the amplified selected target viral RNA sequence and the amplified reference RNA sequence.

191: The process of claim 190, wherein the reference RNA sequence consists of a linear arrangement of a sequence present in the selected target viral RNA sequence, a sequence not present in the selected target viral RNA sequence and a sequence present in the selected target viral RNA sequence.

192: The process of claim 190, wherein the target viral RNA sequence is a human immunodeficiency virus (HIV) RNA sequence or a human cytomegalovirus (HCMV) RNA sequence.

193: The process of claim 190, wherein a primer utilized in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding sequence.

194: The process of claim 190, wherein the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence are measured by measuring (i) the amount of signal obtained from the amplified target viral RNA sequence and (ii) the amount of signal obtained from the amplified reference RNA sequence.

195: The process of claim 194, wherein the amounts of the signals are determined by the use of labeled probes.

196: The process of claim 195, wherein the label is an isotope or a fluorophore.

197: The process of claim 194, wherein the amounts of the signals are determined by the use of labeled primers in the polymerase chain reaction.

198: The process of claim 197, wherein the label is an isotope or a fluorophore.

199: A process for amplification of a target viral RNA and a reference RNA in a sample which comprises:

- (i) selecting a sequence present in the target viral RNA;
- (ii) adding a known quantity of a reference RNA sequence to the sample, wherein the reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence, wherein the reference RNA sequence and the selected target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified selected target viral RNA sequence are distinguishable by size or by probes;

(iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction and then to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence;

(iv) measuring the amounts of the amplified selected target viral RNA sequence and the amplified reference RNA sequence.

200: The process of claim 199, wherein the reference RNA sequence consists of a linear arrangement of a sequence present in the selected target viral RNA sequence, a sequence not present in the selected target viral RNA sequence and a sequence present in the selected target viral RNA sequence.

201: The process of claim 199, wherein the target viral RNA sequence is a human immunodeficiency virus (HIV) RNA sequence or a human cytomegalovirus (HCMV) RNA sequence.

202: The process of claim 199, wherein a primer utilized in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding sequence.

203: The process of claim 199, wherein the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence are measured by measuring (i) the amount of signal obtained from the amplified target viral RNA sequence and (ii) the amount of signal obtained from the amplified reference RNA sequence.

204: The process of claim 203, wherein the amounts of the signals are determined by the use of labeled probes.

205: The process of claim 204, wherein the label is an isotope or a fluorophore.

206: The process of claim 203, wherein the amounts of the signals are determined by the use of labeled primers in the polymerase chain reaction.

207: The process of claim 206, wherein the label is an isotope or a fluorophore.

208: A process for amplification of a target viral RNA sequence and a reference RNA sequence in a sample which comprises:

combining a known quantity of a reference RNA sequence with the sample, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes;

simultaneously subjecting the target viral RNA sequence and the reference RNA sequence to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the target viral RNA sequence and the reference RNA sequence;

measuring the amounts of amplified target viral RNA sequence and amplified reference RNA sequence.

209: The process of claim 208, wherein the reference RNA sequence consists of a linear arrangement of a sequence present in the target viral RNA sequence, a sequence not present in the target viral RNA sequence and a sequence present in the target viral RNA sequence.

210: The process of claim 208, wherein the target viral RNA sequence is a human immunodeficiency virus (HIV) RNA sequence or a human cytomegalovirus (HCMV) RNA sequence.

211: The process of claim 208, wherein a primer utilized in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding sequence.

212: The process of claim 208, wherein the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence are measured by measuring (i) the amount of signal obtained from the amplified target viral RNA sequence and (ii) the amount of signal obtained from the amplified reference RNA sequence.

213: The process of claim 212, wherein the amounts of the signals are determined by the use of labeled probes.

214: The process of claim 213, wherein the label is an isotope or a fluorophore.

215: The process of claim 212, wherein the amounts of the signals are determined by the use of labeled primers in the polymerase chain reaction.

216: The process of claim 215, wherein the label is an isotope or a fluorophore.

217: A process for amplification of a target viral RNA sequence and a reference RNA sequence in a sample which comprises:

combining a known quantity of a reference RNA sequence with the sample, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes;

simultaneously subjecting the target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction and then to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the target viral RNA sequence if present in the sample and the reference RNA sequence;

measuring the amounts of amplified target viral RNA sequence and amplified reference RNA sequence.

218: The process of claim 217, wherein the reference RNA sequence consists of a linear arrangement of a sequence present in the target viral RNA sequence, a sequence not present in the target viral RNA sequence and a sequence present in the target viral RNA sequence.

219: The process of claim 217, wherein the target viral RNA sequence is a human immunodeficiency virus (HIV) RNA sequence or a human cytomegalovirus (HCMV) RNA sequence.

220: The process of claim 217, wherein a primer utilized in the polymerase chain reaction amplification includes a T-7 RNA polymerase binding sequence.

221: The process of claim 217, wherein the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence are measured by measuring (i) the amount of signal obtained from the amplified target viral RNA sequence and (ii) the amount of signal obtained from the amplified reference RNA sequence.

222: The process of claim 221, wherein the amounts of the signals are determined by the use of labeled probes.

223: The process of claim 222, wherein the label is an isotope or a fluorophore.

224: The process of claim 221, wherein the amounts of the signals are determined by the use of labeled primers in the polymerase chain reaction.

225: The process of claim 224, wherein the label is an isotope or a fluorophore.

226-241: (canceled).

242: The process of claim 190, wherein the sequence not present in the selected target viral RNA sequence is about 21 nucleotides in length.

243: The process of claim 199, wherein the sequence not present in the selected target viral RNA sequence is about 21 nucleotides in length.

244: The process of claim 208, wherein the sequence not present in the selected target viral RNA sequence is about 21 nucleotides in length.

245: The process of claim 217, wherein the sequence not present in the selected target viral RNA sequence is about 21 nucleotides in length.

246-248: (canceled).

249: A process for quantitation of a target viral RNA in a sample which comprises:

- (i) selecting a sequence present in the target viral RNA;
- (ii) adding a known quantity of a reference RNA sequence to the sample, wherein the reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence, wherein the reference RNA sequence and the selected target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified selected target viral RNA sequence are distinguishable by size or by probes;
- (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence;

- (iv) measuring the amounts of the amplified selected target viral RNA sequence and the amplified reference RNA sequence; and
- (v) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence.

250: A process for quantitation of a target viral RNA in a sample which comprises:

- (i) selecting a sequence present in the target viral RNA;
- (ii) adding a known quantity of a reference RNA sequence to the sample, wherein the reference RNA sequence comprises a sequence present in the selected target viral RNA sequence and a sequence not present in the selected target viral RNA sequence, wherein the reference RNA sequence and the selected target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified selected target viral RNA sequence are distinguishable by size or by probes;
- (iii) simultaneously subjecting the selected target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction and then to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the selected target viral RNA sequence if present in the sample and the reference RNA sequence;
- (iv) measuring the amounts of the amplified selected target viral RNA sequence and the amplified reference RNA sequence; and
- (v) determining the relative amount of the target viral RNA present in the sample before amplification from the amount of the amplified selected target viral RNA sequence and the amount of the amplified reference RNA sequence.

251: A process for quantitation of a target viral RNA sequence in a sample which comprises:

- combining a known quantity of a reference RNA sequence with the sample, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a

sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes;

simultaneously subjecting the target viral RNA sequence and the reference RNA sequence to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the target viral RNA sequence and the reference RNA sequence;

measuring the amounts of amplified target viral RNA sequence and amplified reference RNA sequence; and

determining the relative amount of the target viral RNA sequence present in the sample before amplification from the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence.

252: A process for quantitation of a target viral RNA sequence in a sample which comprises:

combining a known quantity of a reference RNA sequence with the sample, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes;

simultaneously subjecting the target viral RNA sequence and the reference RNA sequence in the sample first to a reverse transcription reaction and then to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify the target viral RNA sequence if present in the sample and the reference RNA sequence;

measuring the amounts of amplified target viral RNA sequence and amplified reference RNA sequence; and

determining the relative amount of the target viral RNA sequence present in the sample before amplification from the amount of the amplified target viral RNA sequence and the amount of the amplified reference RNA sequence.

253: An amplification reaction mixture for the quantitation of a target viral RNA sequence in a biological sample, said reaction mixture comprising:

- a target viral RNA sequence;

- a known quantity of a reference RNA sequence, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes; and

- an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified.

254: A reverse transcription reaction mixture for reverse transcribing a target viral RNA sequence suspected of being present in a biological sample, said reaction mixture comprising:

- a target viral RNA sequence;

- a known quantity of a reference RNA sequence, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes; and

- an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified for initiating cDNA synthesis to provide a target viral cDNA and a reference sequence cDNA, whereby following reverse transcription the resulting

target viral and reference sequence cDNAs can serve as templates for amplification for providing amplified reference RNA sequence and amplified target viral RNA sequence.

255: A kit for the quantitation of a target viral RNA sequence in a biological sample comprising individual containers which provide:

a known quantity of a reference RNA sequence, wherein the reference RNA sequence comprises a sequence present in the target viral RNA sequence and a sequence not present in the selected target viral RNA sequence, wherein the reference RNA sequence and the target viral RNA sequence can be amplified by the same or different oligonucleotides and wherein following amplification amplified reference RNA sequence and amplified target viral RNA sequence are distinguishable by size or by probes; and

an oligonucleotide primer pair for each of the target viral RNA sequence and the reference RNA sequence to be amplified.

## **IX. EVIDENCE APPENDIX**

Priority to U.S. Serial No. 07/148,959 filed on January 27, 1988 is claimed. A copy of this application was included as Appendix B to the Amendment and Request for Interference Under 37 C.F.R. 1.607 filed on December 18, 1996 in U.S. Serial No. 07/402,450 and is included in this Appendix.

A Declaration Under 37 CFR 1.131(a) is being relied upon by Appellants in this appeal. A copy of this Declaration as filed on December 15, 2005 and entered into the application file is included in this Appendix.

Application Serial No. 07/402,450  
Appellants' Brief on Appeal Under 37 C.F.R. § 41.37  
dated 25 January 2010

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U.S. SERIAL NO. 07/148,959  
FILED ON JANUARY 27, 1988

APPENDIX B

PATENT APPLICATION OF  
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and

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FOR

SIMULTANEOUS AMPLIFICATION  
OF CERTAIN RNA SEQUENCES

SIMULTANEOUS AMPLIFICATION  
OF CERTAIN RNA SEQUENCES.

This invention relates to the detection and quantification of viral RNA from peripheral blood samples or H-9 cells by simultaneous amplification with at least one other RNA sequence.

BACKGROUND OF THE INVENTION

Co-pending Murakawa et al application Serial No. 941,379 filed December 13, 1986 describes a procedure for the amplification of small quantities of viral RNA. The Murakawa procedure comprises adding to RNA two converging oligodeoxyribonucleotide primers at least one of which is complementary to a sequence included in the RNA; thereafter extending at least one of the primers with one or both of reverse transcriptase and DNA polymerase I; and determining whether the sequence is present in the amplified segment by use of a synthetic oligodeoxyribonucleotide probe complementary to a region within the segment. The specification and claims of application Serial No. 941,379 in their entirety are by express reference incorporated herein and made a part hereof.

This invention adapts the Murakawa two primer amplification procedure to the identification and quantification of viral RNA present in peripheral blood samples and H-9 cells. More particularly such RNA is amplified simultaneously with at least one other RNA sequence, the amplification product of which provides a positive control useful to reveal false negative data indicating the absence of viral RNA. The invention includes a kit comprising reagents appropriate for use in its practice.

DETAILED DESCRIPTION OF THE INVENTION

To provide a positive control, at least one synthetic RNA sequence is amplified simultaneously with RNA from a virus affected T-4 lymphocyte or H-9 cell present in a peripheral blood sample.

A first primer pair includes an oligonucleotide sequence effective to amplify viral RNA. For AIDS virus (HIV-1), preferred primers are synthetic oligonucleotides including the following sequences which occupy positions within the HIV-1 3' ORF region:

HIVA: 5' ATGCCGATTGTGCTTGGCTA 3'  
HIVB: 5' TGAATTAGCCCTTCAGTCC 3'

A preferred HIV-1 hybridization probe includes the sequence:  
HIVC (PROBE): 5' AAGTGCTAAGATCTACAAGTGGCT 3'

The process of this invention can also be applied to human cytomegalovirus (HCMV). A target for amplification is a region of the HCMV major IE gene (IE1) region between nucleotides 1254 and 1331. Oligodeoxyribonucleotides complementary to sequences in this region are used with RNA from HCMV infected cells, and with RNA from patient samples. Suitable oligonucleotide primers and probes have the following sequences:

HCMVA	1154	5'	CGAGACACCGGTGACCAAGG	3'	1173
HCMVB	1311	3'	CTCTTTCTACAGGACCGTCT	5'	1330
HCMV (Probe)	1182	3'	AAGGACGTTGTATACAAGTCTT	5'	1204

An additional amplification system is needed for detection of RNA from transcription of late HCMV genes, which is an important marker for active infection. Sequences 865-1025 from the coding sequence of p64 are amplified. Suitable oligonucleotide primers and probes have the following sequences:

HCMVD	866	5'	AAAGAGCCCGACGCTCTACTACAGT	3'	890
HCMVE	1001	3'	CTGGTCATGCACTTCACATGGACC	5'	1025
HCMV Probe II	941	3'	CCGTCCTTCACCAACGAGGTACTCTTG	5'	970

The cell population primarily affected by a virus, e.g., the AIDS virus, is the T-4 lymphocyte population which, like other T-cells express the T-cell receptor.

Accordingly, a second primer pair includes an oligonucleotide to amplify a sequence which is unique to the T-cell receptor. Although other such unique sequences may be selected, preferably the second primer is effective to amplify the constant region of the T-cell receptor beta chain. For HIV-1, T-cell receptor primers A and B are preferred.

T-Cell Receptor A: 5'GTCCACTCGTCATCTCCGA 3'  
T-Cell Receptor B: 5'TCAAGATCCAGATACTGCT 3'

A preferred T-cell receptor hybridization probe includes the following sequence:

T-Cell Receptor C (PROBE): 5'CAGAAGCTGCGCGAGACCTTAGGC 3'

A third primer pair is effective to amplify an RNA sequence present, preferably ubiquitously, in all of the cells of a peripheral blood sample or of an H-9 cell sample, even when the T-cell count is low. Preferably the third primer pair amplifies beta actin sequence. Synthetic oligonucleotides comprising the following sequences are preferred:

Beta Actin A: 5'CTCATTCGCAATGGTGAAGACCTG 3'  
Beta Actin B: 5'GCTATCCCTGTACGCGCTTGGC 3'

A preferred beta actin hybridization probe includes the following sequence:

Beta Actin C (PROBE): 5'CGGTGAGGATCTTCATGAGTAGTC 3'

A fourth primer to provide an additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples.

For HIV-1 such a reference RNA may be a "maxigene" formed by a multi-base pair insert into a unique site, for example the unique KpnI site of the 3' ORF region. A preferred reference RNA includes a 22 base pair insert into the KpnI site of the HIV-1 3' ORF region of the pGEM92 clone described by Murakawa. An insert of sequence: 5'CACACAAGGCTACTTCGGTAC  
CATGTTGTTCGATGAAGC3' is appropriate. The underlined

sequences present in the AIDS virus bracket the 22 base pair insert.

The pGEM92 clone is produced using as a starting material the plasmid pSP64-BH10-R3 (Biotech Research Laboratories, Inc.) which contains the entire HIV virus excluding the LTRs. A 1.1 Kb Sam HI restriction fragment including HIV sequences 6052 to 9149 was subcloned in both orientations into the Sam HI site of the transcription vector pGEM2 (Promega Biotech). The resulting plus strand plasmid is designated pGEM92. The 22 base pair sequence is inserted in known manner.

The transcription product of this clone is 22 bases longer than the authentic HIV-sequence but still hybridizes with the 25 mer probe HIVC. It is therefore distinguishable by size from the authentic viral product.

For purposes of identification and quantification, the amplification products are electrophoresed in a gel, e.g., agarose or 6% polyacrylamide, 8 M. urea gel. Labelled probes complementary to each of the amplified sequences are used sequentially. Hybridization of the probes with amplification products other than of authentic HIV provides positive controls thus minimizing the possibility of false negative data regarding the authenticity of the original sample. More particularly if the HIV probe yields negative data, but one or both the T-cell receptor and beta actin probes yield positive data, the conclusion may be feasibly drawn that the original sample was viable notwithstanding the negative HIV probe result.

The "maxigene" provides an internal control and an additional aid to quantitation. Because the quantity of "maxigene" RNA originally included in the amplification reaction is known, the amount of signal obtained from this amplification product can be related to the signal obtained from the patient sample. Hence, the relative quantitation of the original amount of authentic HIV-1 in the patient sample is also provided by this construct.

To provide appropriate signals either the primers or the probes are labelled, e.g., with an isotope such as P<sup>32</sup> or a fluorescent. Preferably, the probes are labelled.

Amplification using an oligonucleotide primer containing the T-7 RNA polymerase (Biorad Laboratories) increases the sensitivity of detection. The following sequence HIV T7 is illustrative:

HIV T7 - 5'-TTAATACGACTCACTATAGGCGATGCTGATTGTGCTGGCTA 3'

Figure 1 exemplifies simultaneous priming with HIVA and B and T-cell receptor A and B synthetic oligonucleotides. Figure 1A shows the results with HIVC probing. Figure 1B shows the results with T-cell receptor probing.

Figure 2 shows that HIV oligonucleotide primers and probes have homology with the human cytomegalovirus (HCMV) suggesting that HIV-1 has homology with an actively transcribed region of HCMV.

#### EXEMPLIFICATION OF THE INVENTION

##### Example 1

Amplification is performed using LX amplification buffer (10 mM Tris-HCl, pH 7.5; 10 mM MgCl<sub>2</sub>; 66 mM NaCl; 1 mM dithiothreitol), 1.5 mM of each dNTP. To this buffer, about 1 mM total peripheral blood lymphocyte RNA from an AIDS infected patient and about 1.0 mM of each of the priming nucleotides HIVA, HIVB T-cell receptor A and T-cell receptor B

are added providing a final reaction volume of approximately 100  $\mu$ l. The sample is heated at 95°C for 2 minutes, centrifuged for 5 seconds, cooled to 37°C for about 2 minutes at which time 1.0  $\mu$ l of AMV reverse transcriptase (Life Sciences or BioRad Laboratories) diluted in the amplification buffer were added and incubation was continued for 2 minutes at 37°C. A second amplification cycle was performed in like manner. Thereafter the final 28 rounds of amplification were accomplished using a buffer consisting of 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Cetus or New England Biolabs): 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM  $MgCl_2$ , 0.01% gelatin, 200  $\mu$ M each dNTP, and 50 pmoles of each primer in a final volume of 50 microliters overlain with 10 microliters of paraffin oil. The polymerizations are carried out from 1 to 2 minutes at 45°C, with 1 minute of denaturation at 95°C, and 1 minute of annealing at 37°C.

After completion of the last cycle of amplification, the products are placed on ice and a 10  $\mu$ l portion was electrophoresed in a 1.8% agarose gel. The DNA was transferred to Zeta probe (Biorad) using an alkaline blotting procedure and prehybridized and hybridized as follows: The prehybridization reaction was performed at 65°C for 1 to 3 hours in 20 ml of 6X SSPE (1.0 M NaCl, 0.06 M  $NaPO_4$ , 0.005 M EDTA); 1.0% SDS; 0.5% rehydrated, powder skim milk (Alba); and 10  $\mu$ g per ml of sonicated, denatured salmon sperm DNA. The hybridization reaction was in 20 ml of the same buffer, except the salmon sperm DNA was omitted and replaced with 20 pmol of 5'-<sup>32</sup>P-labelled oligodeoxyribonucleotide HIVC (ca.  $3 \times 10^8$  cpm). Hybridization was for 1 hour to overnight at 65°C. The hybridized filter was washed with three 250 ml volumes of 6X SSC (0.95 M NaCl, 0.095 M Na Citrate), 0.1% SDS at 65°C for 3

minutes each, and autoradiographed at -70°C for 1 hour on Kodak  
XAR-S film with an intensifying screen.

Each of the HIVC and T-cell receptor C probes is used  
separately and sequentially. After the results with the HIVC  
probe are obtained, that probe is stripped from the filter by  
treatment with 100C 0.1 X SSC, 0.1% SDS, two times for 15  
minutes each. The filter is then rehybridized to the T-cell  
receptor C probe.

Bands from each of the authentic HIV and T-cell receptor  
samples are detected after Southern Blot hybridization.

EXAMPLE II

Example I is repeated with the exception that the primer  
pair beta actin A and beta actin B is included in the  
amplification reaction mixture.

The amplification products are analyzed separately and  
sequentially by probes which hybridize with authentic viral  
RNA, the amplified T-cell receptor RNA sequence and the  
amplified beta actin A sequence. Bands from each such  
sequence are detected after Southern blot hybridization.

EXAMPLE III

Example I is repeated with the exception that the maxigene  
primer is included in the reaction mixture.

Kits contemplated by the invention include self-contained  
appropriate quantities of primers and probes for use in the  
practice of the invention.

WE CLAIM:

- 1 1. A process for identifying a viral RNA nucleotide sequence  
2 present in a sample of peripheral blood or K-9 cells which  
3 comprises amplifying such RNA simultaneously with at least one  
4 other RNA nucleotide sequence present in a virus infected cell in  
5 said sample, and thereafter separately and sequentially analyzing  
6 the amplification reaction products with probes homologous with  
7 authentic RNA and with such other RNA sequence to identify one of  
8 both of said RNA nucleotide sequences.
- 1 2. The process of claim 1 in which the viral RNA is HIV-1  
2 RNA.
- 1 3. The process of claim 1 in which the viral RNA is HCMV RNA.
- 1 4. The process of claim 1 in which a third RNA nucleotide  
2 sequence ubiquitously present in substantially all of the cells  
3 of said sample is included in the amplification reaction and in  
4 which the analysis of the amplification product includes a third  
5 probe to identify said third nucleotide sequence.
- 1 5. The process of claim 4 in which the viral RNA is HIV-1  
2 RNA.
- 1 6. The process of claim 4 in which the viral RNA is HCMV RNA.

**ABSTRACT**

A process for identifying a viral RNA nucleotide sequence present in a sample of peripheral blood or H-9 cells which comprises amplifying such RNA simultaneously with at least one other RNA nucleotide sequence present in a virus infected cell in said sample, and thereafter separately and sequentially analyzing the amplification reaction products with probes homologous with authentic RNA and with such other RNA sequence to identify one or both of said RNA nucleotide sequences.



FIG. 1A    FIG. 1B  
1A       1B

FIG. 1A - AIDS PROBE  
FIG. 1B - T-CELL RECEPTOR PROBE

H9 Cells infected with AIDS.  
P = AIDS Patient Sample.



FIGURE 2

DECLARATION UNDER 37 CFR 1.131(a)  
FILED ON DECEMBER 15, 2005  
IN U.S. SERIAL NO. 07/402,450



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	: 07/402,450	Confirmation No.:	8131
Applicant	: George J. MURAKAWA et al.		
Filed	: 1 September 1989		
TC/A.U.	: 1631		
Examiner	: Marina Miller		
Attorney Docket No.	: 2124-154		
Customer No.	: 6449		

Director of the United States Patent  
and Trademark Office  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 CFR 1.131(a)**

Dear Sir:

We, George J. MURAKAWA, R. Bruce WALLACE, John A. ZAIA and John J. ROSSI, applicants for the above-identified patent application, declare as follows:

1. That some time on or prior to 21 August 1989, we conceived the idea of quantifying target viral RNA using a known amount of a reference RNA sequence as an internal control. That is, a reference RNA sequence, such as a maxigene or a minigene (each of which contain target viral RNA sequence), was added to the sample containing the target viral RNA. The target viral RNA and the reference RNA sequence were then simultaneously amplified using a polymerase chain reaction. The amounts of the amplified products were measured, and the amount of target viral RNA present before amplification was determined from the amounts of the amplified products.

2. The conception and reduction to practice occurred in the United States of America.

3. The date of conception prior to 21 August 1989 was determined by a draft patent application which describes the invention based on experiments conducted by us. Exhibit I is a

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4. It is further declared that the accompanying exhibits may not be a complete record of applicants' data concerning the invention of the instant patent application and are not necessarily meant to represent the earliest date of conception. The accompanying exhibits are presented solely to prove a completion of the invention prior to the date of Wang et al., U.S. Patent No. 5,219,727, and Wang et al., U.S. Patent No. 5,476,774, listed on the Information Disclosure Statement filed 4 June 2004.

The declarants further state that the above statements were made with the knowledge that willful false statements and the like are punishable by fine and/or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that any such willful false statement may jeopardize the validity of this application or any patent resulting therefrom.

Dated: \_\_\_\_\_

\_\_\_\_\_  
George J. MURAKAWA

Dated: \_\_\_\_\_

\_\_\_\_\_  
R. Bruce WALLACE

Dated: 12-02-05

  
\_\_\_\_\_  
John A. ZAIA

Dated: \_\_\_\_\_

\_\_\_\_\_  
John J. ROSSI



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.	: 07/402,450	Confirmation No.:	8131
Applicant	: George J. MURAKAWA et al.		
Filed	: 1 September 1989		
TC/A.U.	: 1631		
Examiner	: Marina Miller		
Attorney Docket No.	: 2124-154		
Customer No.	: 6449		

Director of the United States Patent  
and Trademark Office  
P.O. Box 1450  
Alexandria, VA 22313-1450

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Dated: \_\_\_\_\_

\_\_\_\_\_  
George J. MURAKAWA

Dated: Dec. 1, 2005

R. Bruce Wallace  
R. Bruce WALLACE

Dated: \_\_\_\_\_

\_\_\_\_\_  
John A. ZALA

Dated: \_\_\_\_\_

\_\_\_\_\_  
John J. ROSSI



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George J. MURAKAWA

Dated: \_\_\_\_\_

\_\_\_\_\_  
R. Bruce WALLACE

Dated: \_\_\_\_\_

\_\_\_\_\_  
John A. LAMA

Dated: \_\_\_\_\_

\_\_\_\_\_  
John J. ROSSI

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Exhibit 1



METHOD FOR AMPLIFICATION  
AND DETECTION OF RNA SEQUENCES

This application is a continuation-in-part of each of application Serial No. 353,296 filed May 22, 1989, (which is a continuation-in-part of application Serial No. 941,379, filed December 15, 1986), application Serial No. 143,045 filed January 12, 1988 (which is a continuation-in-part of application Serial No. 941,379), and application Serial No. 148,959 filed January 27, 1988. Application Serial Nos. 353,296, 143,045 and 148,959 are incorporated in this application by reference.

BACKGROUND

It is known to utilize the polymerase chain reaction (PCR) to amplify RNA and DNA sequences present in small samples. The amplification procedure can be simultaneously performed on more than one sequence in the same sample. The presence or absence of a specific sequence in the amplification products may be determined by oligonucleotide hybridization assays. See generally Mullis, U.S. patent 4,683,195.

Virus etiology generally and retrovirus etiology in particular is complex. See Varmus, Retroviruses, Science 240:1427-1435 (1988). Known PCR techniques, as applied rapidly diagnose or confirm potential retroviral positive patients, are of limited sensitivity, lack positive controls and may otherwise be unreliable. For example, persons who were seropositive but both virus culture-negative and PCR-negative are reported by Ou et al. Science 233:293-297 (1988). As a first explanation for this observation, Ou suggests that these persons may have contained an insufficient number of provirus copies to be directly detected by the PCR technique utilized.

*Spencer →*

SUMMARY OF THE INVENTION

This invention provides a PCR technique of improved sensitivity and which ~~may~~ include positive controls for determination of the presence or absence of a target sequence in viral RNA sample.

Increased sensitivity is provided by utilizing viral RNA as the original PCR template. The viral RNA is converted to complementary DNA which is then amplified.

The invention is useful to detect unique sequences in samples containing as few as 100 molecules of RNA. It can be used to detect retroviral RNA in samples from as little as 10 nanograms (ng) of total cellular RNA.

The practical application of the invention to clinical diagnosis of virus infected patients is apparent. Patients who harbor a viral genome but are not yet producing anti-viral antibodies may be diagnosed as uninfected by known screening methods. The invention enables detection of viral transcripts, such as those of the AIDS virus which may accumulate in the absence of viral protein translation during the early stages of infection. Clinical applications of the invention include the identification and quantification of viral RNA present in peripheral blood samples and ~~cells~~. *Laboratory cell line*

DETAILED DESCRIPTION OF THE INVENTION

In general the method of the invention entails utilizing a sample RNA which has or may have a target viral sequence as a template for amplification by PCR. A first oligonucleotide primer for the target viral sequence is annealed to the template for extension through the target sequence to produce a first extension product having an RNA template strand and a DNA primer extension strand. The first extension product is denatured and the separated RNA template and DNA primer extension strands are

annealed, respectively to the first primer and to a second primer complementary to the DNA primer extension strand. The first and second primers are positioned for extension through the target sequence on the template and its complement on the primer extension strand. The first and second primers are extended to produce a second primer extension product which is denatured, the first and second primers are again annealed to the separated template and primer extension strands, and again extended and the resulting extension products denatured. The process is repeated for the number of cycles deemed appropriate to achieve the desired degree of amplification.

After the final round of amplification and denaturation, the product is analyzed, for example, by oligonucleotide hybridization assay to determine the presence or absence of a sequence indicative of the presence of the target sequence in the sample.

In the early cycles, e.g., the first five cycles after the production and denaturing of the first extension product, the amplification steps are conducted in the presence of both reverse transcriptase and the large fragment of <sup>D</sup>RNA polymerase I (Klenow) or similarly functioning enzyme. Subsequent cycles may appropriately be conducted in the absence of reverse transcriptase. Ribonuclease A is preferably added after about 5 to about 7 cycles of DNA amplification *to destroy residual RNA and reduce its ability to compete as the template.*  
In the preferred practice of the invention, both the first and second primer are present throughout the amplification procedure. Alternatively the second primer can be added at any stage of the process prior to the amplification of the denatured first extension product.

The process of the invention is useful to amplify and detect viral RNA from any source. It has particular application to the

detection and quantification of AIDS (HIV-1) virus and  
cytomegalovirus (HCMV).

For AIDS virus amplification and detection preferred first  
and second primers are synthetic oligonucleotides having the  
following sequences:

HIVA: 5'ATGCCGATTGTGCTTGCTA 3'

HIVE: 5'TGAATTAGCCCTTCCAGTCC 3'

A preferred HIV-1 hybridization probe includes the sequence:

HIVC (PROBE): 5'AAGTGGCTAGATCTACAGCTGCCT 3'

As applied to human cytomegalovirus (HCMV), a target for  
amplification is a region of the HCMV major IE gene (IE1) region  
between nucleotides 1154 and 1331. Oligodeoxyribonucleotides  
complementary to sequences in this region are used with RNA from  
HCMV infected cells, or from patient peripheral blood samples.  
Suitable oligonucleotide primers and probes have the following  
sequences:

HCMV	1154 5'	CGAGACACCCGTCACCAAGG	3' 1173
HCMV	1311 3'	CCTCTCTACAGGACGCTCT	5' 1330
HCMV (Probe I)	1182 3'	AAGGACGCTCTATACACTCCTT	5' 1204

An additional amplification system is needed for detection of  
RNA from the transcripts of late HCMV genes, which are  
important markers for active infection. For this purpose,  
sequences 866-1025 from the coding sequence of p64<sup>L</sup> may be  
amplified. Suitable oligonucleotide primers and probes have the  
following sequences:

HCMV	866 5'	AAAGAGCCCGACGCTCTACTACACCT	3' 890
HCMV	1001 3'	CTGGTCATGCACTTCCACATGGACC	5' 1025
HCMV Probe II	941 3'	CGCGTGCCTGACCAACAGGATCTCTTG	5' 976

#### EXAMPLE I

This example illustrates the amplification of in vitro  
synthesized RNA by the use of the plasmid pSP64-BH10-R3 (Biotech  
Research Laboratories, Inc.), containing the entire HTLV-III

1/ Fuger, B., et al. J. Virol. 61:446 (1987).

(HIV-1) virus excluding the LTRs, as the starting material for the following subclone vectors. A 1.1 kb BamHI restriction fragment including HIV-1 sequences 8052 to 9149 was subcloned in both orientations into the BamHI site of the transcription vector pGEM2 (Promega Biotec). The resulting plasmids, pGM92 (+ strand) and pGM93 (- strand), were digested with EcoRI and transcribed with T7 RNA polymerase using a T7 transcription kit (BioRad Laboratories, Inc.).

10<sup>-1</sup> pmol of RNA from pGM92 was subjected to 4, 5, 8 and 10 cycles of amplification. Amplification was performed using IX <sup>1</sup> amplification buffer (10 mM tris-HCl, pH 7.5; 10 mM MgCl<sub>2</sub>; 66 mM NaCl; 1 mM dithiothreitol), 1.5 mM of each dNTP, and 1.0 μM of each oligodeoxyribonucleotide (HIVA and HIVR, supra) in a final reaction volume of 100 μl. Samples were denatured by heating to 95°C for 2 minutes, spun in a microfuge for 5 seconds, cooled to 37°C for 2 minutes, at which time 1.0 μl of reverse transcriptase (2.0 units, BioRad), diluted in amplification buffer, was added for 2 minutes. Cycles 2-5 were performed as described above, except both reverse transcriptase and Klenow (0.5 units, Boehringer Mannheim) were added. In cycle 6, RNase A was added (0.45 μg) and only DNA pol I was used. All subsequent cycles of amplification were performed with only the presence of DNA pol I. After completion of the last cycle of amplification, samples were placed on ice and a 10.0 μl portion was electrophoresed in a 1.8% agarose gel. The DNA was transferred to Zeta probe (BioRad) using an alkaline blotting procedure<sup>2</sup> and prehybridized and hybridized as follows: The prehybridization reaction was performed at 65°C for 1 to 3 hours in 20 μl of 6X SSPE (1.0 M NaCl, 0.06 M NaPO<sub>4</sub>, 0.006 M EDTA); 1.0% SDS; 0.5% rehydrated,

11/ Southern, S.W. J. Mol. Biol. 58 503-517 (1975).

Reed & Mann - rev DNA prep  
-5-

powder skim milk (~~ATPS~~); and 10 ug per ml of sonicated, denatured salmon sperm DNA. The hybridization reaction was in 20 ml of the same buffer, except the salmon sperm DNA was omitted and replaced with 20 pmol of 5'-<sup>32</sup>P-labelled oligodeoxyribonucleotide probe HIVC (ca.  $3 \times 10^8$  cpm). Hybridization was for 1 hour to overnight at 65°C. The hybridized filter was washed with three 250 ml volumes of 6X SSC (0.95 M NaCl, 0.095 M Na Citrate), 0.1% SDS at 65°C for 5 minutes each, and autoradiographed at -70°C for 1 hour on Kodak XAR-5 film with an intensifying screen.

Densitometric scanning and integration, *up the peak area* amplification were performed. This revealed a 3.81 fold level of amplification. Thus, if 21 cycles were performed with this template, and since only one strand is synthesized during the first cycle, we calculate the theoretical amplification would be over 400,000 fold.

#### EXAMPLE II

To test the sensitivity of amplification, samples in which  $10^{-9}$ ,  $10^{-7}$ , and  $10^{-5}$ , pmol of pGM92 RNA were used in repetitions of Example I. After 21 cycles of replication, bands from each of the samples can be detected after Southern blot hybridization. *Since only one leg of the reaction was used* the detection of the sample from only  $10^{-9}$  pmol, *this result shows* that as few as 100 molecules of RNA are *possible no more in a sample* sufficient for detection after amplification.

#### EXAMPLE III

This Example demonstrates amplification of an RNA template in the presence of non-specific RNA. 5.5 ug of bovine rRNA was added to a reaction mixture as described in Example I containing  $10^{-3}$  pmol of GM92 RNA. Specific amplification was seen at high efficiency.

EXAMPLE IV

This Example demonstrates that RNA isolated from HIV infected cells can be efficiently utilized for amplification and detection pursuant to this invention. Polymerase chain reaction using only 10 ng of total RNA from HIV infected H9 cells was performed as described in Example I. A specific hybridizing band, about two orders of magnitude lower than the 1.0 ug sample, was observed. To test if the amplification of the in vivo sample was from RNA or residual DNA contamination, a control sample in which RNase A was added prior to amplification was examined. In this experiment, no hybridization band was detected after prolonged autoradiographic exposure.

For identification and quantification purposes it is preferred to amplify the viral RNA sample simultaneously with at least one other RNA sequence to provide a positive control. In this way the risk of false negative data indicating the absence of viral RNA in, for example, peripheral blood and H-9 cell samples may be reduced.

To provide such positive controls at least one synthetic RNA sequence is amplified simultaneously with the RNA sample. In clinical practice the sample usually is from virus infected T-4 lymphocytes of ~~which cells~~ present in a peripheral blood sample. Such embodiments entail use of a plurality of first and second primer pairs. One pair is provided for each RNA sequence to be amplified. The amplification procedure is otherwise accomplished as previously described.

The cell population primarily affected by a virus, e.g., the AIDS virus, is the T-4 lymphocyte population which, like other T-cells express the T-cell receptor.

17  
18  
19  
Accordingly, a suitable second primer pair includes oligonucleotides to amplify a sequence which is unique to the T-cell receptor. Although other such unique sequences may be selected, preferably such a primer is effective to amplify the constant region of the beta chain of the relevant virus T-cell receptor. For HIV-1, T-cell receptor the following primers A and B are preferred:

T-Cell Receptor A: 5'CTCCACTCGTCATCTCCGA 3'  
T-Cell Receptor B: 5'TCAGAGCTCCAGATAGCT 3'

A preferred T-cell receptor hybridization probe includes the following sequence:

T-Cell Receptor C (PROBE): 5'CAGAAGGTGGCCGAGACCCCTCAGGC 3'

Another primer pair is effective to amplify an RNA sequence present, preferably ubiquitously, in all of the cells of a patent sample such as a peripheral blood sample or of ~~a T-cell~~ <sup>sample</sup>, even when the T-cell count is low. Preferably such primer pairs amplify beta actin sequence. Synthetic oligonucleotides comprising the following sequences are preferred:

Beta Actin A: 5'CTCATTTGCCAATGGTCATGACCTG 3'  
Beta Actin B: 5'GCTATCCCTGTACGCTCTGGC 3'

A preferred beta actin hybridization probe includes the sequence:

Beta Actin C (PROBE): 5'CGGTGAGGATCTTCATGAGGTAGTC 3'

An additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples.

Such a reference RNA may be a "minigene" or a "maxigene" formed by a multi-base pair insert into or deletion from a unique site. For example a preferred reference RNA includes a 22 base pair insert into the KpnI site of the HIV-1 3' <sup>het-</sup> ~~env~~ region of the pGEM32 clone described in Example I. An insert of sequence:

5' CACACR AG GCTAC 77 CGGTAC  
 3' GACACAAGCTACCTGCTAC CATGGTGTTCGACATGACG 5' is appropriate.

The underlined sequences present in the AIDs virus bracket the 22 base pair insert.

The transcription product of this clone is 22 bases longer than the authentic HIV-sequence but still hybridizes with the 25-mer probe HIVC. It is therefore distinguishable by size from the authentic viral product.

Such "minigenes" and "maxigenes" provide an internal control and an additional aid to quantitation. Because the quantity of "maxigene" RNA originally included in the amplification reaction is known, the amount of signal obtained from this amplification product can be related to the signal obtained from the patient sample. Hence, the relative quantitation of the original amount of authentic HIV-1 in the patient sample is also provided by this construct.

Similar procedures can be used as a quantitative assay of HCMV sequences. A segment of the cDNA derived from the major IE gene IE1 is subcloned into the transcription vector pTZ18U (BioRad), and includes nucleotides 1185-1331. A small insertion accomplished either by cloning or by site directed mutagenesis is made in this segment which permits distinction between the PCR-amplified viral RNA and cellular amplified transcripts. By including a fixed amount of this plasmid HCMV RNA or DNA in every sample to be amplified, it is possible to measure the amount of viral DNA or RNA using the in vitro sample as an internal standard.

To provide appropriate signals either the primers or the probes are labelled, e.g., with an isotope such as  $P^{32}$  or a ~~fluorescent~~ <sup>fluorophore</sup>. Preferably, the probes are labelled.

Amplification using an oligonucleotide primer containing the T-7 RNA polymerase (BioRad Laboratories) increases the sensitivity of detection. *When the amplification is followed*  
The following HIV T7 sequence is illustrative:

HIV T7 - 5' *T-7* TTAATAGGACTTCACCTATAGGATGCTGATTGTGCGTGGCTA 3' *from*

For purposes of identification and quantification, the *Me 5*  
amplification products may be electrophoresed in a gel, e.g., agarose or 6% polyacrylamide, 7 M. urea gel. Labelled probes complementary to each of the amplified sequences are used sequentially. Hybridization of the probes with amplification products other than of authentic viral sequence, e.g., HIV or HCMV provides positive controls thus minimising the possibility of false negative data regarding the authenticity of the original sample. More particularly, if the authentic, e.g., HIV probe yields negative data, but one or both the T-cell receptor and beta actin probes yield positive data, the conclusion may be feasibly drawn that the original sample was viable notwithstanding the negative HIV probe result.

EXAMPLE IV *U*

Amplification is performed using 1X amplification buffer (10 mM Tris-HCl, pH 7.5; 10 mM MgCl<sub>2</sub>; 66 mM NaCl; 1 mM dithiothreitol), 1.5 mM of each dNTP). To this buffer, about 1 mM total peripheral blood lymphocyte RNA from an AIDS infected patient in about 1.0 mM of each of the priming nucleotides HIVA, HIVB T-cell receptor A and T-cell receptor B are added providing a final reaction volume of approximately 100 µl. The sample is heated at 95°C for 2 minutes, centrifuged for 5 seconds, cooled to 37°C for about 2 minutes at which time 1.0 µl of AMV reverse transcriptase (Life Sciences or BioRad Laboratories) diluted in the amplification buffer were added and incubation was continued for 2 minutes at 37°C. A second amplification cycle was

performed in like manner. Thereafter the final 28 rounds of amplification were accomplished using a buffer consisting of 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Cetus or New England Biolabs): 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM  $MgCl_2$ , 0.01% gelatin, 200  $\mu$ M each dNTP, and 50 pmoles of each primer in a final volume of 50 microliters overlain with 10 microliters of paraffin oil. The polymerizations are carried out from 1 to 2 minutes at 65°C, with 1 minute of denaturation at 95°C, and 1 minute of annealing at 37°C.

After completion of the last cycle of amplification, the products are placed on ice and a 10  $\mu$ l portion was electrophoresed in a 1.8% agarose gel. The DNA was transferred to Zeta probe (BioRad) using an alkaline blotting procedure and prehybridized and hybridized as follows: The prehybridization reaction was performed at 65°C for 1 to 3 hours in 20 ml of 6X SSPE (1.0 M NaCl, 0.06  $NaPO_4$ , 0.006 M EDTA); 1.0% SDS; 0.5% rehydrated, powder skin milk (Alba); and 10  $\mu$ g per ml of sonicated, denatured salmon sperm DNA. The hybridization reaction was in 20 ml of the same buffer, except the salmon sperm DNA was omitted and replaced with 20 pmol of 5'- $^{32}P$ -labelled oligodeoxynucleotide HIVC (ca.  $3 \times 10^8$  cpm). Hybridization was for 1 hour to overnight at 65°C. The hybridized filter was washed with three 250 ml volumes of 6X SSC (0.95 M NaCl, 0.095 M Na Citrate), 0.1% SDS at 65°C for 5 minutes each, and autoradiographed at -70°C for 1 hour on Kodak XAR-5 film with an intensifying screen.

Each of the HIVC and T-cell receptor C probes is used separately and sequentially. After the results with the HIVC probe are obtained, that probe is stripped from the filter by treatment with 100C 0.1 X SSC, 0.1% SDS, two times for 15 minutes each. The filter is then rehybridized to the T-cell receptor C probe.

Bands from each of the authentic HIV and T-cell receptor samples are detected after Southern Blot hybridization.

Figure 1 exemplifies simultaneous priming with HIVA and B and T-cell receptor A and B synthetic oligonucleotides. Figure 1A shows the results with HIVC probing. Figure 1B shows the results with T-cell receptor probing.

~~Figure 2 shows that HIV oligonucleotide primers and probes have homology with the human cytomegalovirus (HCMV) suggesting that HIV-1 has homology with an actively transcribed region of HCMV.~~

#### EXAMPLE V

Example I is repeated with the exception that the primer pair beta actin A and beta actin B is included in the amplification reaction mixture.

The amplification products are analyzed separately and sequentially by probes which hybridize with authentic viral RNA, the amplified T-cell receptor RNA sequence and the amplified beta actin A sequence. Bands from each such sequence are detected after Southern blot hybridization.

#### EXAMPLE VI

Example I is repeated with the exception that the maxigene primer is included in the reaction mixture.

Kits contemplated by the invention include self-contained appropriate quantities of primers and probes for use in the practice of the invention.

A typical kit for the detection and quantification of HIV-1 virus in a patient peripheral blood sample includes vials or similar separate containers filled with, for example, 1 ml of each of HIV-1 A and HIV-1 B in 1 % solution in 1 % solution and 1 ml of HIV-1 C in 1 % solution. Reference RNA in 1 % solution is included in an additional container. Such kits

X

20 primers/microtiter (as stored H<sub>2</sub>O)  
each of HIVA, HIVB or HIVC. A reference RNA  
in 14000 copies/microtiter in prepared  
should DEPC treated water

include reagents and instructions necessary to conduct the  
appropriate amplification and hybridization procedures.

WE CLAIM:

1. The process for detecting the presence or absence of a viral RNA in a sample which comprises

(i) utilizing the sample as a template for hybridization with a first primer for a target viral RNA sequence present in substantially all transcripts of a virus, said primer being present in excess and positioned for extension through said target sequence;

(ii) extending said first primer to provide a double stranded first extension product having an RNA template strand including said target sequence and a DNA primer extension strand complementary to said target sequence;

(iii) denaturing said first extension product to provide a mixture containing said first primer and said separate RNA template and DNA primer extension strands;

(iv) providing, in the step (iii) mixture a second primer for extension through said sequence in said DNA primer extension strand which is complementary to said target sequence;

(v) annealing said first and second primers respectively to said separated RNA template strands and DNA primer extension strands;

(vi) extending said annealed primers;

(vii) denaturing the extension products produced in step (vi);

(viii) subjecting the product of step (vii) to hybridization with a labelled oligonucleotide probe for said target viral sequence or for a sequence complementary to said target sequence.

2. A process as defined by claim 1 in which steps (v), (vi), and (vii) are repeated prior to step (viii).

3. A process as defined by claim 1 or claim 2 in which said second primer is present during steps (i), (ii), and (iii).

4. A process as defined by claims 1, 2, or 3 in which said viral RNA is human immunodeficiency virus (HIV) RNA or human cytomegalovirus (HCMV).

5. A process as defined by claims 1, 2 or 3 for detecting the presence or absence of viral RNA in a human H-9 cell or peripheral blood sample.

6. A process as defined by claims 1, 2, or 3 for detecting the presence or of HIV-1 or HCMV in a human H-9 cell or peripheral blood sample.

7. A process as defined by claims 1, 2, or 3 in which said viral RNA is HIV-1 and in which

(i) said target viral sequence is the BamHI-Sst-I fragment within the 3' <sup>761</sup>ORF region of the HIV-1;

(ii) said first primer includes the sequence  
5'TGAATTAGCCCTTCAGTCC encoding nucleotides 8677 to 8688.

(iii) said second primer includes the sequence  
5'ATGCTGATTGTGCTGGCTA, encoding nucleotides 8538 and 8547,  
and

(iv) said probe includes the sequence  
3'AAGTGGCTAAGATCTACAGTGCTCT, encoding nucleotides 8642 to 8648.

8. The process for detecting the presence or absence of a human cytomegalovirus (HCMV) in a sample which comprises:

(i) utilizing the sample as a template for hybridization with a first primer for a target viral RNA sequence present in all transcripts of said HCMV, said primer being present in excess and being positioned for extension through said target sequence;

(ii) extending said first primer to provide a double stranded first extension product having an RNA template strand including said target sequence and a DNA primer extension strand including a sequence complementary to said target sequence;

(iii) denaturing said first extension product to provide a mixture containing said first primer and separate RNA template and DNA primer extension strands;

(iv) providing in the step (iii) mixture a second primer for extension through said sequence in said DNA primer extension strand which is complementary to said target sequence;

(v) annealing said first and second primers respectively to said separated RNA template strands and DNA primer extension strands;

(vi) extending said annealed primers;

(vii) denaturing the extension products produced in step (vi);

(viii) adding a labelled oligonucleotide probe for said target viral sequence to the product of step (vii).

9. A process as defined by claim 8 in which steps (v), (vi) and (vii) are repeated prior to step (viii).

10. A process as defined by claim 8 in which said second primer is present during steps (i), (ii) and (iii).--

11. A process as defined by claim 8 in which said first primer includes the sequence 5' CGAGACACCGTGACCAAGG 3' encoding nucleotides 1154 to 1173;

said second primer includes the sequence

3' CTCCTTCACAGGACCGTCT 5' encoding nucleotides 1311 to 1330, and

said probe includes the sequence

3' AAGGACGTCTGATCAACTCCTT 5'.

12. A process as defined by claim 8 for detecting the presence or absence of RNA from a human cytomegalovirus late gene transcript in a sample in which said target viral sequence includes nucleotides from the coding sequence of p64, said first primer includes the sequence 5' AAGAGCGCCGACGTCTACTACCGT 3', said second primer includes the sequence 3'

CGCGTGTCTCGACCAAAACGAGGTACCTCTTG 5', and said probe includes the sequence 3' CGCGTGTCTCGACCAAAACGAGGTACCTCTTG 5'.

13. A process which comprises:

(i) providing an amplification reaction mixture of a viral RNA, including a target sequence, a primer for annealing to said target sequence, reverse transcriptase, and a second primer for the annealing to and for extending across the DNA complement of said RNA target sequence;

(ii) annealing said first primer to said target sequence;

(iii) extending said first primer across said target sequence to provide a first extension product having an RNA target sequence strand and a DNA primer extension strand;

(iv) denaturing said first extension product to provide separate RNA target sequence and DNA primer extension strands;

(v) annealing said first and second primers to said separate RNA target sequence strands and DNA primer extension strands;

(vi) extending said annealed primers to produce a second extension product;

(vii) denaturing said second extension product;

(viii) repeating steps (ii) through (vii);

(ix) subjecting the amplification product after step (viii) to hybridization with a labelled oligonucleotide probe for said target sequence or for a sequence complementary to said target sequence.

14. A process as defined by claim 1 or 2 in which

(a) at least one synthetic RNA sequence which does not include said target sequence of which includes substantially more or less nucleotides than said target sequence is subjected, with said sample to polymerase chain reaction

amplification under conditions appropriate to simultaneously amplify said target sequence and said reference sequence;

(b) the amplification products of step (a) are denatured and thereafter separately and sequentially subjected to hybridization conditions with oligonucleotide probes for said target sequence and said reference sequence.

15. A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood or H-9 cell sample which comprises:

- (i) selecting said target viral RNA sequence;
- (ii) simultaneously subjecting
  - (a) said sample and
  - (b) at least one synthetic RNA sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence

to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification products produced by step (ii);

(iv) subjecting said denatured amplification products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subsection of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences hybridized with said probes homologous therewith.

16. A process as defined by claim 15 in which the reference nucleotide sequence utilized in step (ii) is

(i) a sequence present in the T-cell receptor expressed by cells affected by the virus containing said viral RNA;

(ii) a preselected RNA sequence present in substantially all of the cells of said sample,

(iii) a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence;

(iv) a beta actin sequence.

17. A process as defined by claim 15 in which said target viral sequence is located within the 3' ORF region of HIV-1 and in which the reference sequence utilized in step (ii) is located in the constant region of the beta chain of the T-cell receptor expressed T-cells affected by HIV-1.

18. A process as defined by claim 17 in which the reference sequence utilized in step (ii) is a beta actin sequence.

19. A process as defined by claim 17 in which the reference sequence utilized in step (ii) is a sequence formed by inserting a multi-base pair sequence into the 3' ORF region of HIV-1.

20. A process as defined by claim 15 or 17 in which at least one of the primers utilized in conducting the polymerase chain reaction in step (ii) includes the T-7 RNA polymerase sequence.

21. A process as defined by claim 15 in which said target viral sequence is in the HCMV major immediate early (IE) gene.

22. A process as defined by claim 15 in which said target viral sequence comprises RNA from the transcription of late HCMV genes.

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## **X. RELATED PROCEEDINGS APPENDIX**

The decisions noted in Section II above are included in this Appendix. Copies of these decisions were taken from the file of the present application.

REMAND TO EXAMINER  
APPEAL NO. 93-4018  
APPLICATION NO. 07/402,450  
MAILED AUGUST 6, 1996

PAPER NO. 32 IN U.S. SERIAL NO. 07/402,450

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today  
(1) was not written for publication in a law journal and (2) is  
not binding precedent of the Board.

Paper No. 32

UNITED STATES PATENT AND TRADEMARK OFFICE

MAILED

AUG 08 1996

BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES

PAT. & T.M. OFFICE  
BOARD OF PATENT APPEALS  
AND INTERFERENCES

Ex parte GEORGE J. MURAKAWA, R. BRUCE WALLACE,  
JOHN A. ZAIA, and JOHN J. ROSSI

Appeal No. 93-4018  
Application No. 07/402,450<sup>1</sup>

ON BRIEF

Before WILLIAM F. SMITH, JOHN D. SMITH, and GRON, Administrative  
Patent Judges.

WILLIAM F. SMITH, Administrative Patent Judge.

REMAND TO THE EXAMINER

We remand this application to the examiner for consideration  
of the following issues.

<sup>1</sup> Application for patent filed September 1, 1989.  
According to applicants (specification, page 1), the application  
is a continuation-in-part of (1) 07/355,296, filed May 22, 1989,  
(2) 07/143,045, filed January 12, 1988, and (3) 07/148,959 filed  
January 27, 1988. (1) and (2) are each a continuation-in-part of  
06/941,379, filed December 15, 1986.

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I.

The Examiner's Answer contains a provisional rejection of claims 18 through 25 and 31 through 33 under 35 U.S.C. § 102(e) over application 07/180,740.<sup>2</sup> The provisional § 102(e) rejection was not previously made in any Office action and therefore constituted a new ground of rejection, although it was not labeled as such.<sup>3</sup> Accordingly, appellants have not been provided an opportunity to respond to this rejection.

Upon return of this application, the examiner is to reconsider the provisional rejection under § 102(e) based upon application 07/180,740.<sup>4</sup> If that application is no longer pending, the examiner should ascertain whether it has been refiled under 35 U.S.C. § 120. If an anticipation issue remains,

---

<sup>2</sup> The examiner included claims 26 through 30 in this rejection even though they had been cancelled in Paper No. 10, filed December 4, 1991.

<sup>3</sup> Appellants appear to be unaware that this new ground of rejection was made in the answer since the reply brief filed May 7, 1993, which was denied entry, does not acknowledge or respond to the provisional rejection.

<sup>4</sup> The propriety of such a provisional rejection was addressed in Ex parte Bartfeld, 16 USPQ2d 1714, 1716 (Bd. Pat. App. & Int. 1990) aff'd on other grounds, In re Bartfeld, 925 F.2d 1450, 17 USPQ2d 1885 (Fed. Cir. 1991) where the board noted that an important function of provisional § 102(e) rejections is to "put [an applicant] on notice at the earliest possible time of the possible prior art relationship between copending application."

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the provisional rejection should of course be maintained. If it is maintained, however, the examiner should provide appellants with an appropriate opportunity to respond. If the provisional rejection is not maintained, the examiner should explain clearly why it has been overcome.

## II.

The Examiner's Answer contains a second new ground of rejection to which appellants were not permitted to respond. In the final Office action mailed March 19, 1992, claims 18 through 30 were provisionally rejected for obviousness-type double patenting over claims 24 through 53 of application 07/180,740 (Paper No. 13, page 6). In the Examiner's Answer, this provisional rejection was applied to claims 18 through 25 and 31 through 33 and the basis of the rejection was switched to claims 57, 58 and 60 of the 07/180,740 (Paper No. 24, page 5). The switch in the basis of this provisional rejection constituted a new ground of rejection to which appellants should have been given an opportunity to respond.

In addition, the Examiner's Answer does not adequately explain the provisional obviousness-type double patenting rejection. First, the Examiner's Answer contains no explanation

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for the change in the group of claims to which this ground of rejection is applied. Second, the Examiner's Answer contains an inadequate explanation of the relationship between the subject matter of the provisionally rejected claims and the claims of application 07/180,740. The Examiner states only that "both inventions [i.e., that of instant claims 18 through 25 and 31 through 33 and that of claims 57, 58 and 60 of application 07/180,740] are directed to detect an RNA virus via PCR methodology with primers and probes of conserved transcript sequences that are known in the prior art." Examiner's Answer, page 5. This statement also appears in the final Office Action (page 6), where the provisional rejection was over claims 24 through 53 of application 07/180,740. The examiner acknowledges that claims 24 through 53 of application 07/180,740 "have been canceled and amended by the new claims [57, 58, and 60]" (Examiner's Answer, page 8), yet maintains the provisional rejection without any explanation of why the replacement and amendment of the previously problematic claims in application 07/180,740 was ineffective in overcoming the provisional rejection.

Upon return of this application, the examiner is to reconsider the provisional obviousness-type double patenting

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rejection. If the provisional rejection is maintained, the examiner should provide appellants with the required opportunity to respond to it. If it is not maintained, the examiner should clearly explain why it has been overcome.

### III.

The amendments to claim 18 which were submitted December 1, 1992, and which were entered by the examiner, introduce several sources of apparent ambiguity and indefiniteness into claim 18, and by dependency into all of the claims on appeal. First, part (iv) of claim 18 recites hybridizing an amplified product to a probe and "denaturing resulting hybridization product." Part (v) recites hybridizing "the product of step (iv)" with another probe. It is unclear whether the hybridization of step (v) is intended to be carried out with the "hybridization product" recited in step (iv) or with the product that results from carrying out the recited denaturation step on the "hybridization product."

Second, part (vi) recites "determining . . . whether said reference sequences hybridized with said reference sequence." No previous step recites a step of hybridizing the reference sequence to itself. Also, part (vi) is grammatically confusing.

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Third, the results recited to be indicative of false negative data are confusing. Lines 26-28 of claim 18 specify that the "first probe" is homologous to the target sequence, and lines 37-39 recite that "false negative data [are] indicated by failure of either of said first and second probes to hybridize to the sample sequence or to the reference sequence." The wording of the claim implies that false negative data are indicated if either of the probes fails to hybridize to either of the (target or reference) sequences; e.g., by failure of the target probe to hybridize to the reference sequence. But the record indicates that this is not the intended meaning. See, e.g., the chart on page 2 of the Appeal Brief: false negative data are indicated only when the target probe fails to hybridize to the target sequence and when the reference probe fails to hybridize to the reference sequence, but not when either one separately fails to hybridize to its complementary sequence. The chart does not indicate what effect on the assay result would be indicated by failure of each probe to hybridize to the other probe's complementary sequence. The inconsistencies between the claim wording and the assay actually described in the specification causes confusion when interpreting the scope and content of the subject matter claimed.

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Finally, the claim contains several apparent typographical errors. These include at least lines 28-29: "said reference sequence[, ] denaturing"; line 29: "denaturing resulting [sic, the resulting] hybridization product"; line 34: "sequence are"; lines 34-36: "hybridized with said first probe whether [sic, and whether] said reference sequences hybridized."

Upon return of the application, the examiner should review all pending claims for compliance with the definiteness requirement of 35 U.S.C. § 112, second paragraph. As set forth in In re Zletz, 893 F.2d 319, 321-22, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989):

An essential purpose of patent examination is to fashion claims that are precise, clear, correct, and unambiguous. Only in this way can uncertainties of claim scope be removed, as much as possible, during the administrative process.

#### IV.

Several prior art issues also require further attention from the examiner. First is the issue of the effective filing date for each of the claims on appeal. There is no indication in the record that the examiner has considered whether any claim(s) is(are) entitled to the benefit of the earlier filing date of any of the purported parent applications under 35 U.S.C. § 120. We

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note in this regard that the relationship of the parent cases with each other and with the application on appeal is unclear from the specification and the data which appears on the face of the administrative file. The specification states (page 1, lines 3-9) that the application on appeal is

a continuation-in-part of each of application Serial No. 355,296 filed May 22, 1989, (which is a continuation-in-part of application Serial No. 941,379, filed December 15, 1986), application Serial No. 143,045 filed January 12, 1988 (which is a continuation-in-part of application Serial No. 941,379), and application Serial No. 148,959 filed January 27, 1988 (emphasis added).

The face of the file, on the other hand, indicates that the application on appeal is a CIP (continuation-in-part) of 07/355,296, which is a CIP of 06/941,379 and a CIP of 07/143,045, which is a CIP of 07/148,959. The application relationship on the face of the file differs from that in specification in that: (1) the file face indicates that 07/355,296 is a CIP of 07/143,045, while the specification indicates that the application on appeal is a CIP of 07/143,045, and (2) the file face indicates that 07/143,045 is a CIP of (later-filed) 07/148,959, while the specification indicates that the application on appeal is a CIP of 07/148,959.

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Neither appellants nor the examiner have addressed this problem or attempted to reconcile the conflicting statements. However, even that would not have cured the problem since both the specification and the face of the file are wrong since application 07/355,296 is a file-wrapper continuation, not a continuation-in-part, of application 06/941,379. Upon return of the application, the examiner should first ascertain the correct relationship of the purported parent applications and this application. Then the examiner should make sure that the face of the file and the specification recite the correct information.

Subsequently, the examiner should determine the effective filing date of each claim. See In re van Langenhoven, 458 F.2d 132, 136-37, 173 USPQ 426, 429 (CCPA 1972) where the court stated that "subject matter which is first disclosed in a continuation-in-part application is not entitled to the filing date of the parent application" and "[a]s to given claimed subject matter, only one effective date is applicable." After fixing the filing date of each claim, the examiner should reevaluate the prior art since it is not clear which possible filing date served as the basis for the prior art search.

Turning to the merits of the prior art rejections, the examiner's position as understood is that it would have been

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obvious to use a positive control during PCR amplification of viral RNA because positive controls were well known to people skilled in the art at the time. It is apparent from the claim language, however, that the claimed process requires a specific positive control reaction: simultaneous amplification of two distinct sequences (target and reference) during the PCR reaction.

No mention of positive controls was made in any statement of the obviousness rejections before the Examiner's Answer. See, e.g., the final Office Action, page 5 ("it would have been obvious to one of ordinary skill in the art at the time of the instant invention to apply the PCR technique of Mullis et al. to HIV amplification and detection") and page 6 ("as applied above, it would have been obvious to someone of ordinary skill in the art at the time of the instant invention to apply the PCR technique of Mullis et al. to HMCV [sic, HCMV] amplification and detection").

In the Examiner's Answer, the examiner amplifies on the conclusions of obviousness recited in the Office actions, stating on page 4:

It would have been obvious to one of ordinary skill in the art at the time of the instant invention to apply the PCR technique of Mullis et al. to HIV amplification and

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detection . . . . Even though, that Mullis et al. teach simultaneous amplification of more than one sequence at a time at column 10, lines 47-57. The use of an internal standard or control with a measurement or detection technique has been known since quantitative analysis was first practiced. In view of this, one of ordinary skill in the art would have found it prima facie obvious to use a second sequence of RNA to be co-amplified as an internal control with the reasonable expectation, if not certainty, of distinguishing false positive and false negative results.

The examiner does not refer to any of the cited references to support the statement that positive controls were notoriously well known in the art. But even assuming arguendo that (1) positive controls in general were well known and (2) it would have been obvious to use a positive control in any diagnostic reaction, it is apparent that any number of possible reactions could be run to provide a positive control for a PCR reaction. For example, a separate reaction mixture could be "spiked" with a known amount of the viral RNA being assayed for and then subjected to the same PCR reaction as the test sample. The examiner's statement of the rejection begs the question of why it would have been obvious to run the particular positive control described in the claims; i.e., why would it have been obvious to modify Mullis as required to arrive at the claimed process?

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On return of this application, the examiner should reconsider the basis of the obviousness rejection in light of these comments and in light of other references, if any, found to be prior art during determination of the effective filing date of the claims. If the obviousness rejection is maintained over the current references or modified, the examiner should restate the obviousness rejection to indicate clearly what teachings from the prior art would have made the claimed process as a whole obvious. If the obviousness rejection is withdrawn, the examiner should indicate why the positive control reaction recited in the claims is unobvious over positive control reactions the examiner has asserted are well known in the art.

V.

Finally, there is a question regarding the circumstances of the filing of the application. The cover sheet of the initial application submission (September 1, 1989) indicates that no drawings were filed at that time even though the specification as originally filed referred to Figures 1 1A and 1B at page 12. An amendment was filed December 5, 1991 (Paper No. 12), stating that Figures 1, 1A, and 1B were inadvertently omitted from the

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original filing, and requesting deletion of the drawing descriptions from the specification.

The Manual of Patent Examining Procedure (Sixth Edition, Rev.1, Sept. 1995) (MPEP) provides that:

Applications filed without all figures of drawing described in the specification are not given a filing date since they are "prima facie" incomplete. The filing date is the date on which the omitted figures are filed. . . . If any applicant believes that omitted figures of an application are not necessary for an understanding of the subject matter sought to patented, applicant may petition to have the application accepted without the omitted figures. Any such petition must be accompanied by the petition fee (37 CFR 1.17(h)) and an amendment cancelling from the specification all references to the omitted figures . . . . Also, if the oath or declaration for the application was filed prior to the date of the amendment and petition, the amendment must be accompanied by a supplemental declaration by the applicant stating that the invention is adequately disclosed in, and a desire to rely on, the application as thus amended for purposes of an original disclosure and filing date."

MPEP § 608.02.

Thus, the MPEP sets out four requirements that must be met for an application filed without its drawings to receive a filing date: 1) a petition, 2) the petition fee, 3) cancellation of references to the drawings, and 4) a supplemental declaration. Of these requirements, only the requirement of an amendment

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canceled references to the drawings has been met. Until the requirements of a petition, petition fee, and supplemental declaration are met, the application is prima facie incomplete and therefore cannot be accorded a filing date.

On return of this application, the examiner should notify appellants of the steps they must take to complete the application.

The application, by virtue of its "special" status, requires immediate action. See MPEP § 708.01(d). It is important that the Board of Patent Appeals and Interferences be informed promptly of any action affecting the appeal.

## REMANDED

William F. Smith  
WILLIAM F. SMITH  
Administrative Patent Judge

JOHN D. SMITH  
Administrative Patent Judge

BOARD OF PATENT  
APPEALS AND  
INTERFERENCES

Teddy S. Gron.  
TEDDY S. GRON  
Administrative Patent Judge

Application Serial No. 07/402,450  
Appellants' Brief on Appeal Under 37 C.F.R. § 41.37  
dated 25 January 2010

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Appeal No. 93-4018  
Application No. 07/402,450

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MEMORANDUM OPINION AND ORDER

(Decision on Wang preliminary motion 1)

INTERFERENCE NO. 105,055

DATED NOVEMBER 5, 2003

PAPER NO. 49 IN U.S. SERIAL NO. 07/402,450  
(Paper No. 36 in Interference No. 105,055)

The opinion in support of the decision being  
entered today is not binding precedent of the Board

49  
Paper

By: Trial Section Merits Panel  
Board of Patent Appeals and Interferences  
U.S. Patent and Trademark Office  
P.O. Box 1450  
Alexandria, VA 22313-1450  
Tel: 703-308-9797  
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Filed:  
5 November 2003

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES  
(Administrative Patent Judge Carol A. Spiegel)

ALICE M. WANG, MICHAEL E. DOYLE  
and DAVID F. MARK

Junior Party,  
U.S. Patent 5,219,727  
U.S. Patent 5,476,774

v.

GEORGE J. MURAKAWA, R. BRUCE WALLACE,  
JOHN A. ZAIA and JOHN J. ROSSI

Senior Party,  
Application 07/402,450

Patent Interference No. 105,055

Before: SCHAFFER, TORCZON and SPIEGEL, Administrative Patent Judges.  
SPIEGEL, Administrative Patent Judge.

MEMORANDUM OPINION and ORDER  
(Decision on Wang preliminary motion 1)

Interference No. 105.055  
Wang v. Murakawa

Paper 36  
Page 2

Before us for consideration is Wang's preliminary motion 1 pursuant to 37 CFR § 1.633(a) that all the involved Murakawa claims, i.e., claims 34-35, 38-39 and 42-47, are unpatentable under 35 U.S.C. § 135(b)(1). We grant Wang's motion and remand the case to the APJ for further proceedings not inconsistent with this opinion.

#### I. Introduction

This interference concerns a PCR<sup>1</sup>-based method for determining the amount of a target nucleic acid sequence in a sample by simultaneously amplifying the target nucleic acid sequence and a known amount of an added internal standard nucleic acid sequence ("control sequence"<sup>2</sup>) with the same oligonucleotide primer pair in a single

---

<sup>1</sup> PCR or "polymerase chain reaction" is an in vitro method for enzymatically synthesizing or "amplifying" a target nucleic acid (DNA) sequence. The reagents typically required for PCR include (a) a DNA polymerase, (b) each of the four nucleotide building blocks of DNA (i.e., dNTPs A, T, G and C), (c) a source of template DNA, such as double-stranded DNA containing the target sequence, and (d) two primers designed to be complementary to the bases at the 3' ends of the target DNA sequence.

Amplification of the target sequence occurs in repeated cycles of three defined steps called denaturation, annealing and primer extension. A reaction solution is prepared by adding (c) an excess of primer, (b) all four nucleotides (A, T, G and C) and (a) a DNA polymerase to (c) a source of template DNA, e.g., double-stranded DNA. In the first step, the double-stranded DNA is heated near boiling to separate the paired strands of DNA into single strands. In the second step, the temperature is lowered to allow the primers to anneal or hybridize to their complementary DNA sequences on the single strands produced in the previous step (because of the large excess of primers, the two separated DNA strands will bind to the primers instead of to each other). In the third step, the DNA polymerase produces complementary copies of the initial single strands of DNA from the primers hybridized to the DNA. That is, primer annealing produces a nucleic acid molecule that is partially double-stranded where the primer has hybridized and partially single-stranded where the primer has not hybridized. The DNA polymerase uses the double-stranded annealed primer portion as a substrate and sequentially adds a nucleotide to the 3' end of the primer which is complementary to the nucleotide which is "across" from it on the single-stranded portion of the nucleic acid molecule to produce a "primer extension product."

The three steps are repeated. The original and newly synthesized double-stranded DNA are separated. In this way the double-stranded products of the previous cycle become new templates for the next cycle such that at each cycle the amount of the target sequence flanked by the primers essentially doubles. Assuming a 100% efficiency at each cycle, one copy of a target sequence in a sample would be increased to about a million copies after 20 cycles.

<sup>2</sup> In this decision, the terms "standard," "reference" and "control" are equivalent when used in reference to the added internal standard nucleic acid. Furthermore, the term "predetermined quantity" is equivalent to the term "predetermined amount" in this decision.

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Page 3

reaction mixture (Count 1). This interference also concerns a plasmid<sup>3</sup> having a 5' sequence and a 3' sequence which provide upstream and downstream primer hybridization sites that are identical to the 3' and 5' primer hybridization sites on a target DNA (Count 2), such that the plasmid is useful as a control sequence.

## **II. Findings of fact (FF)**

The following findings of fact are supported by a preponderance of the evidence.

1. The junior party is ALICE M. WANG, MICHAEL D. DOYLE and DAVID F. MARK (Wang).
2. Wang is involved in the interference on the basis of two patents:
  - (i) U.S. Patent 5,219,727 ("the Wang 1993 patent"), which issued June 15, 1993, based on application 07/413,623, filed September 28, 1989, and
  - (ii) U.S. Patent 5,476,774 ("the Wang 1995 patent"), which issued December 19, 1995, based on application 08/028,464, filed March 9, 1993.
3. Claims 1-4 and 6-10 of the Wang 1993 patent correspond to Count 1 in this interference.
4. Claims 5-7, 10-12 and 15-18 of the Wang 1995 patent correspond to Count 1 in this interference. Claims 1-3 and 8-9 of the Wang 1995 patent correspond to Count 2 in this interference.
5. The senior party is GEORGE J. MURAKAWA, R. BRUCE WALLACE, JOHN A. ZAIA and JOHN J. ROSSI (Murakawa).

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<sup>3</sup> A plasmid is a small, circular, self-replicating DNA molecule found in bacteria.

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6. Murakawa is involved in the interference on the basis of application 07/402,450 ("Murakawa '450"), filed September 1, 1989.
7. Murakawa '450 has been accorded benefit for the purpose of priority of
  - (i) application 07/143,045 ("Murakawa '045"), filed January 12, 1988, and
  - (ii) application 07/148,959 ("Murakawa '959"), filed January 27, 1988.
8. Claims 34-35, 38-39 and 42-47 of Murakawa '450 correspond to Count 1 in this interference. Claim 45 of Murakawa '450 corresponds to Count 2 in this interference.
9. Pursuant to 37 CFR § 1.607, Murakawa requested an interference with the 1995 Wang patent (Ex. 2023).
10. According to Murakawa,

The patent which claims subject matter which interferes with subject matter claimed in the present application is U.S. patent no. 5,476,774 (the "'774 patent") issued on December 19, 1995 to Wang et al. . . . The '774 patent was issued from Ser. No. 028,464, filed March 9, 1993, which purports on its face to be a continuation of Ser. No. 413,623, filed September 28, 1989 (now U.S. patent no. 5,219,727), . . . [id., p. 4.]

\* \* \* \* \*

The '774 patent issued on December 19, 1995, which is less than one year prior to the filing of the present claims/request. Therefore, the provisions of 35 U.S.C. 135(b) have been satisfied. [id., p. 7.]

Other findings of fact follow below.

### III. The legal standard

Section 135(b)(1) of 35 U.S.C. states that "[a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted." To establish entitlement to the earlier effective

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date of existing claims for purposes of the one-year bar of 35 U.S.C. § 135(b), a party must show that the later filed claim does not differ from an earlier claim in any "material limitation." Corbett v. Chisholm, 568 F.2d 759, 765-66, 196 USPQ 337, 343 (CCPA 1977). "Inclusion of a limitation in a claim to avoid the prior art provides strong evidence of the materiality of the included limitation. Parks v. Fine, 773 F.2d 1577, 1579, 227 USPQ 432, 434 (Fed. Cir. 1985)." In re Berger, 279 F.3d 975, 982, 61 USPQ2d 1523, 1527 (Fed. Cir. 2002). "If all material limitations of the copied claims are present in, or necessarily result from, the limitations of the prior claims, then the copied claim is entitled to the earlier effective date of those prior claims for purposes of satisfying 35 U.S.C. § 135(b)." Id., 279 F.3d at 982, 61 USPQ2d at 1527.

**IV. The Wang 1993 patent claims contain two material limitations -- (1) use of predetermined initial amount of a control sequence and (2) use of a shared primer pair for amplifying the control and target sequences**

11. In an Office action mailed October 21, 1991 (Ex. 2015, pp. 3<sup>4</sup> and 5), claims 1-27 of the application that matured into the Wang 1993 patent were rejected under 35 U.S.C. § 103 as unpatentable over Chelly et al., "Transcription of the dystrophin gene in human muscle and non-muscle tissues," Nature, 333:858-860 (June 30, 1988) (Ex. 2009).

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<sup>4</sup> According to the Examiner,

Chelly et al. teach a method of quantitating a target nucleic acid using co-amplification of the target and an internal standard nucleic acid. The Chelly et al. teaching differs ... in ... using a gene different from the target as the standard; this gene binds primers which are different from those bound by the target. [Ex. 2015, p. 3, penultimate ¶.]

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12. Wang argued that

...Chelly fails to disclose two critical elements ... The first missing element is the use of a single pair of primers to amplify both target and standard polynucleotides. ... the second missing element is a known concentration of a standard. ... Without knowledge of the original quantity of standard present, there is no way of using PCR to quantify the amount of target polynucleotide in a sample. [Ex. 2016, para. bridging pp. 4-5.]

13. The next Office action, mailed May 6, 1992, maintained the rejection of kit claims 17-19 of the application that matured into the Wang 1993 patent over Chelly et al. (Ex. 2017, pp. 4-5).

14. In response, Wang amended claim 17<sup>5</sup> and argued that

... Chelly is missing two elements of the present invention. First, Chelly does not provide that a single pair of primers can amplify both a segment of the standard and a segment of the target sequence. ... Second, ... Chelly did not provide a known quantity of standard. [Ex. 2018, para. bridging pp. 21-22.]

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...Chelly does not provide an internal standard nucleic acid and primers able to amplify both a segment of the internal standard nucleic acid and a segment of the target; therefore, Chelly does not provide the elements of the kit of claim 17 and those claims dependent thereon ... [Id., p. 24, para. 2, original emphasis.]

15. The final Office action, mailed October 30, 1992, withdrew the rejection of the claims over Chelly (Ex. 2019).

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<sup>5</sup> A marked up copy of amended claim 17 reads as follows (brackets indicated deletions; underlining indicates additions):

17. (Amended) A kit for the quantitation of a [particular] target nucleic acid segment in a biological sample comprising individual containers which provide: a predetermined initial amount of an internal standard nucleic acid segment for quantitation of a target nucleic acid; and [at least one] an oligonucleotide primer pair wherein said primer pair can serve to amplify a nucleic acid segment contained within the internal standard together with a segment contained within the particular target nucleic acid [Ex. 2018, pp. 3-4.]

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16. In the Notice of Allowability, mailed January 8, 1993, the Examiner's Statement of Reasons for Allowance stated that "[t]he closest prior art (Chelly et al) uses, as a standard, a nucleic acid that binds a different set of primers than the target and therefore teaches away from use of a standard which will bind the same primers as the target" (Ex. 2020, p. 2, last para.).

17. The Wang 1993 patent issued on June 15, 1993 with one independent and nine dependent method claims. Independent claim 1 reads (emphasis added):

A method for quantifying a target nucleic acid segment in a sample, which method comprises the steps of:

(a) **adding to said sample a predetermined initial amount of standard nucleic acid segment wherein said standard nucleic acid segment binds to same primers as are bound by said target nucleic acid segment** in a reaction mixture;

(b) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said nucleic acids are rendered single-stranded and exposed to an agent for polymerization, deoxynucleoside 5'-triphosphates, and a pair of oligonucleotide primers, wherein **said pair of primers is specific for both the target and standard nucleic acid segments**, such that an extension product of each primer of said pair can be synthesized using separate strands of the target and standard segments as a template for synthesis, such that the extension product of one primer, when it is separated from the template strand, can serve as a template for the synthesis of the extension product of the other primer of said pair wherein said amplified target and standard segments are distinguishable by size or by the use of internal probes, wherein said internal probes may be differentially labeled for each of said amplified target and standard segments;

(c) separating the primer extension product from the templates on which they were synthesized to form single-stranded molecules;

(d) repeating steps (b) and (c) on the single stranded molecules produced in step (c) at least one, whereby each repeat of steps (b) and (c) is one amplification cycle;

(e) measuring the amounts of the amplified target and standard segments produced in step (d); and

(f) calculating from the amplified target and standard segments produced in step (d) the amount of said target nucleic acid segment present in the sample before amplification.

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Wang contends that the claims of the Wang 1993 patent contain three material limitations -- (1) the use of predetermined initial amount of a control sequence, (2) the use of a shared primer pair for amplifying the target and control sequences, and (3) the placement of the control sequence on a plasmid (Paper 26, pp. 8-10, 14 and 19). Murakawa maintains that the earlier Murakawa claims contain the asserted material limitations (1) and (2), and that (3) is not a material limitation (Paper 35, p. 1).

We agree with Murakawa that the placement of the control sequence on a plasmid is not a material limitation of the Wang 1993 claims for three reasons. First, Wang has not pointed to, and we do not find, any rejections, including prior art rejections, during the prosecution of either the Wang 1993 or 1995 patents which were overcome by amending the claims with a "plasmid" limitation. Second, Henry A. Erlich, Ph.D., Wang's own expert witness, admits that it is not a critical limitation, i.e., having a shared primer pair amplify the target and control sequences is critical, not limiting the control sequence to a plasmid (Ex. 1010, p. 31, l. 10 - p. 33, l. 18).<sup>6</sup> Third, in its reply

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<sup>6</sup> Dr. Erlich testified upon cross-examination as follows (Ex. 1010, p. 31, l. 10 - p. 33, l. 18):

- Q: ... The fact that there's a plasmid claimed is not a material limitation, is it?
- A: I don't think so. The material limitation referred to in [paragraph] 16 [of Dr. Erlich's Declaration, Ex. 2011] is the shared primer pair.
- Q: OK. So the fact that it's -- it's a shared primer pair, that's important. And the fact that the shared primer pair will also amplify a plasmid isn't critical, is that correct?
- A: The crucial element is having the shared primer pair amplified the reference sequence as well as the target sequence. That's the crucial element.
- Q: It's not crucial that the reference be on a plasmid. It could be in any form, correct?
- A: I think that's correct.
- \*\*\*\*\*
- Q: OK. But either generating the reference either way work be it a plasmid or synthetically, correct?
- A: In principle, they should be both able to work as a control or reference or standard sequence.

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(Paper 34, pp. 4-5), Wang recharacterized the issue as "whether any of the Murakawa claims (i.e., those filed or pending prior to the § 135(b) critical date) necessarily resulted in those two material limitations", i.e., a predetermined initial amount of a reference nucleic acid, and the use of a shared primer pair.

#### **V. The earlier Murakawa claims**

In determining the level of ordinary skill in the art, we considered several factors, including the educational level of the inventors, the sophistication of the technology, the educational level of active workers in the art and the content of the prior art of record. In our opinion, Henry A. Erlich, Ph.D.<sup>7</sup> and Gerald F. Joyce, M.D., Ph.D.,<sup>8</sup> are persons of ordinary skill in the art.

18. The critical date for purposes of 35 U.S.C. § 135(b)(1) is June 15, 1994, one year after the issue date of the 1993 Wang patent.

##### **A. Earlier Murakawa claims recite use of predetermined initial amount of a control sequence**

19. Involved Murakawa independent reaction mixture claims 34, 35, 46 and 47 and kit claim 44 all recite having "a predetermined initial amount of a control sequence" (Ex. 2010). Murakawa claims 34, 35 and 44 were initially presented on December 18, 1996

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<sup>7</sup> Dr. Erlich testified for Wang that, in his opinion, "a person of ordinary skill in the art of PCR in 1988 and '89 was a person with a Ph.D. degree or equivalent experience in genetics, molecular biology, or similar technology and at least two years of laboratory experience with PCR" (Ex. 2011, ¶ 5).

<sup>8</sup> Gerald F. Joyce, M.D., Ph.D., testified for Murakawa that, in his opinion, "a person of ordinary skill in the art of PCR in 1988 and 1989 was person with an undergraduate degree in the life sciences or chemistry, a graduate degree in molecular biology, biochemistry or a closely related discipline, and at least one year of post-doctoral experience. If the person's graduate degree was not in molecular biology or biochemistry, his or her graduate school course work and laboratory experience would have included significant contributions from these sciences. The person would have had one to two years experience with PCR." [Ex. 1004, ¶ 5.]

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(Ex 2023) and subsequently amended. Murakawa claims 46 and 47 were initially presented on December 19, 1996 (Ex 2029) and subsequently amended. None of the involved Murakawa claims had been presented or were pending as of June 15, 1994, the critical date.

20. Wang and Murakawa both agree that (i) Murakawa '959 claims 15-17 and 20 (Ex. 2027,<sup>9</sup> p. 3; Ex. 2028,<sup>10</sup> p. 2) and (ii)(a) Murakawa '450 claims 26-28 and 30, as originally filed September 1, 1989 (Ex. 2006) and (ii)(b) claims 31-33, added on December 4, 1991 (Ex. 2007), use a predetermined quantity of a control sequence. [See Murakawa admission in Paper 35, p. 3 of Wang's statement of material fact 20 in Paper 26, p. 8.]

Use of a predetermined initial amount of a control sequence is explicitly recited in (i) Murakawa '959 claim 15 (and claims 16,17 and 20, dependent thereon, (Ex. 2027, p. 3; Ex. 2028, p. 2)) and (ii)(a) Murakawa '450 original claim 26 (and claims 27-28 and 30, dependent thereon (Ex. 2006)) and (ii)(b) claim 31 (and claims 32 and 33, dependent thereon, added on December 4, 1991 (Ex. 2007)).

21. Murakawa '959 claim 15 reads (Ex. 2027, pp. 1-3, emphasis added):

A process as defined in claim 7

[A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood or H-9 cell sample which comprises

- (i) selecting said target viral RNA sequence;
- (ii) simultaneously subjecting (a) said sample and (b)

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<sup>9</sup> Murakawa "Amendment" filed February 27, 1989 in Murakawa '959.

<sup>10</sup> Murakawa "Amendment Responsive to Final Action Mailed July 3, 1989" filed July 25, 1989 in Murakawa '959.

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at least one reference nucleotide sequence which does not include said target sequence or which contains substantially more nucleotides than said target sequence;  
to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification products produced by step (ii);

(iv) subjecting the denatured amplification products of step (iii) to hybridization conditions separately and sequentially with oligonucleotide probes homologous to said target sequence and to said reference sequence;

whereby hybridization of the probe with the amplified reference sequence provides a positive control and minimizes the possibility of false negative data]

**wherein a predetermined quantity of the reference sequence is utilized in step (ii)(b);** the probes utilized in step (iv) are labelled; and, the presence or absence of the reference sequence in the denatured amplification products of step (iii) is detected in step (iv) by southern blot hybridization with said labelled oligonucleotide probes.

22. Murakawa '450 original claim 26 read (Ex. 2006, p. 21, emphasis added):

A process as defined in claim 18

[A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood cell sample which comprises:

(i) selecting said target viral RNA sample;

(ii) simultaneously subjecting (a) said sample and (b) at least one synthetic RNA sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sample;

(iii) denaturing the amplification products produced by step (ii);

(iv) subjecting said denatured amplification products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence

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with which it hybridized prior to the separate and sequential separation of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences hybridized with said probes homologous therewith]

**wherein a predetermined quantity of the reference sequence is utilized in step (ii)(b); the probes utilized in step (iv) are labelled; and, the presence or absence of the target sequence and the presence of the reference sequence in the denatured amplification products of step (iii) is detected in step (iv) by Southern blot hybridization with said labelled oligonucleotide probes.**

23. Murakawa '450 claim 30, as of December 1, 1991, read (Ex. 2007, pp. 1-2):

A process as defined by claim 18

[A process for discerning false negative data or false positive data in the identification of a target viral RNA sequence in a peripheral blood cell sample which comprises:

(i) selecting said target viral RNA sequence;

(ii) simultaneously subjecting (a) said sample and (b) at least one synthetic RNA reference sample which does not include said target sequence or which includes substantially more nucleotides than said target sequence or which includes at least about 20 nucleotides less than said target sequence to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification product or products produced by step (ii);

(iv) subjecting said denatured amplification product or products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subjection of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences are hybridized with said probes homologous therewith, false negative data being indicated

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by failure of said probes to hybridize either to the sample or to the reference sequence and false positive data being indicated by hybridization of the target sequence probe and by the absence of hybridization of the reference sequence probe]

**wherein a predetermined quantity of said reference sequence is used in step (ii)(b) and the probes utilized in step (iv) are labelled.**

**B. Earlier Murakawa claims do not require or necessarily result in use of a shared primer pair for amplifying control and target sequences**

24. Involved Murakawa independent reaction mixture claims 34, 35, 46 and 47 and kit claim 44 all require an oligonucleotide primer pair which can serve to amplify said control sequence and said target nucleic acid (i.e., viral RNA) sequence (Ex. 2010).

25. According to Dr. Erlich,

...use of a primer pair is conventional in PCR. That is, in order for PCR to proceed, the reaction requires a primer at each end of the two ends of the sequence to be amplified. It is important to distinguish between the use of different pairs of primers for the control sequence and the target sequence such as taught by Chelly *et al.* (Exhibit 2009) from the use of a shared primer pair for both the control and target sequences. None of the earlier Murakawa claims<sup>11</sup> recite the inclusion or use of a shared primer pair. Moreover, such a shared primer pair does not necessarily arise from the limitations of those earlier Murakawa claims. That is, for example, the subject matter of the earlier Murakawa claims all can be practiced using primer pairs that are not shared between the control sequence and the target sequence. [Ex. 2011, ¶ 12.]

Murakawa disagrees, alleging that Wang's recitation of "a standard nucleic acid segment that 'binds to [the] same primers' as are bound by the target nucleic acid segment" is the same as Murakawa's reference "to amplifying a reference sequence

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<sup>11</sup> The "earlier" Murakawa claims refer to filed or pending by June 15, 1994, i.e., claims 1-33 as they existed as of June 15, 1994 (Exs. 2006-2008) and to the claims of the earlier Murakawa applications (Exs. 2004-2006, 2021-2022 and 2024-2028) (Ex. 2011, ¶ 6).

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and a target sequence simultaneously by PCR" when Murakawa's claims are construed by one skilled in the art in light of the Murakawa specifications (Paper 35, pp. 16-17). Specifically, Murakawa argues that Murakawa '450 original claim 18, by virtue of reciting a Markush group expressly including a sequence which includes substantially more nucleotides than the target sequence, necessarily results in the use of a "shared primer pair" (Paper 35, pp. 21-22). Murakawa further argues that Murakawa '450 original claim 30 "when properly construed, requires a known amount of an identifiable reference sequence that binds the same primer pair as the target sequence, and further requires that the reference sequence be amplified by PCR in the presence of (and therefore simultaneously with) the target sequence" (*id.*, p. 22)

Thus, to prevail, Murakawa must show that the earlier Murakawa claims not only require a predetermined initial amount of a control sequence, but also require or necessarily result in the use of a shared primer pair.<sup>12</sup>

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<sup>12</sup> On cross-examination, Dr. Joyce testified as follows:

Q: As a part of preparing your declaration did you try to find a single claim within the Murakawa applications that's directed exclusively to the combination of the two material limitations in the Wang claims for quantitation?

A: Did I try to? I would say, no, I didn't try to. But I would have noticed the extent to which a claim pertained to the so-called material limitations in the case.

Q: Did you find a single claim that was directed exclusively to the combination of the two material limitations in connection with quantitation?

A: No, I did not.

[Ex. 2030, p. 19, l. 25 - p. 20, l. 12.]

.....

Q: So as you sit here today you're not aware of any claim that's directed exclusively to quantitation containing those two material limitations in the Murakawa cases?

A: Any single claim?

Q: Yes.

A: Correct.

Q: So what you've done as part of coming up with with [sic] your opinion is to combine various claims together to come up with what you believe contains those two material limitations?

A: Yes.

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26. Murakawa '450 original claim 18 reads (Ex. 2006, p. 19, emphasis added),

A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood cell sample which comprises:

- (i) selecting said target viral RNA sequence;
- (ii) simultaneously subjecting
  - (a) said sample and
  - (b) **at least one synthetic RNA sequence** which does not include said target sequence or **which includes substantially more nucleotides than said target sequence**

to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence<sup>13</sup>;

- (iii) denaturing the amplification products produced by step (ii);
- (iv) subjecting said denatured amplification products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence, each of said probes being removed from a sequence with which it hybridized prior to the separate and sequential subsection of said amplification products to hybridization with another of said probes;
- (v) determining whether said amplified target and reference sequences hybridized with said probes homologous therewith.

27. Murakawa '450 original claim 19 reads (Ex. 2006, p. 20, emphasis added),

A process as defined by claim 18 in which the reference sequence utilized in step (ii) is

- (i) a sequence present in the T-cell receptor expressed by cells affected by the virus containing said viral RNA;
- (ii) a preselected RNA sequence present in substantially all of the cells of said sample;
- (iii) **a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence;**
- (iv) a beta actin sequence.

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[Id., p.20, l. 25 - p. 21, l. 11.]

<sup>13</sup> Murakawa '450 original claim 18 lacks antecedent basis for "said reference sequence." For purposes of this discussion we interpret the --at least one synthetic RNA sequence-- as "said reference sequence."

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28. Murakawa '450 original claim 30 has been reproduced above (FF 22) and recites, in relevant part,

(ii) simultaneously subjecting (a) said sample and (b) **at least one synthetic RNA reference sample** which does not include said target sequence or **which includes substantially more nucleotides than said target sequence or which includes at least about 20 nucleotides less than said target sequence** to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence.

In other words, the issue is whether simultaneously subjecting a sample and any of three possible control sequences, i.e.,

- (1) "at least one synthetic RNA sequence ... which includes substantially more nucleotides than said target sequence" (Murakawa '450 original claim 18),
- (2) "a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence" (Murakawa '450 original claim 19), or
- (3) "at least one synthetic RNA reference sample ... which includes substantially more nucleotides than said target sequence or which includes at least 20 nucleotides less than said target sequence" (Murakawa '450 original claim 30),

to PCR amplification under conditions appropriate to simultaneously amplify a target sequence, if present, and the control sequence necessarily results in or requires use of shared primer pairs.

**1. claims 18, 19 and 30 do not exclude use of shared primer pairs**

29. Obviously, as Dr. Joyce testified for Murakawa, "[i]f the reference sequence and the target sequence have the same primer binding sites, both the target sequence and the reference sequence will be amplified simultaneously by PCR when the same primer pair, i.e., the shared primer pair, is used" (Ex. 1004, ¶ 34).

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However, as noted by Wang in its reply (Paper 34, p. 6), Dr. Joyce merely states what would happen under such conditions, not that simultaneous amplification of the target and reference sequences require use of a shared primer pair.

30. For example, Dr. Joyce also testified that "[i]t is possible to fall within ... claim [18] and not use shared primer pairs" (Ex. 2030, p. 23, ll. 1-5).

**2. claims 18, 19 and 30 do not require or necessarily result in use of shared primer pairs**

Here, the exact nature and sequence of the three aforementioned possible control sequences are not stated in Murakawa '450 claims 18, 19 and 30. Rather, they are described as (i) having "substantially more" nucleotides than the target sequence or (ii) including "a multi-base insertion" at a "preselected" site in the target sequence or (iii) having "at least 20 nucleotides less" than the target sequence. Neither the size of the target sequence (claim 18) nor the size of the insert (claim 19) is specified.

Pending claims are given the broadest reasonable interpretation consistent with applicant's specification. *In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989). Furthermore, while claims are read in light of the specification, specification limitations are not read into the claims. *Id.*, 893 F.2d at 320-21, 13 USPQ2d at 1322.

**a. "substantially more," "multi-base insertion" and "preselected" site are not terms of art**

31. Here, Drs. Joyce and Erlich both agree that, in this context, the terms "substantially more", "multi-base insertion" and a site "preselected" with respect to said target sequence do not have ordinary and customary meanings to one skilled in the art. [Ex. 1004, ¶¶ 24-25; Ex. 1010, p. 23, ll. 8-25, p. 24, l. 24 - p. 25, l. 4.]

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- i. **a control sequence that contains "substantially more" nucleotides than the target sequence, but does not contain the target sequence, cannot use a shared primer pair**

Claim 18 does not require its control sequence to include the target sequence.

32. Indeed, Dr. Joyce testified that claim 18 encompasses a control sequence that contains "substantially more" nucleotides than a target sequence, but does not contain any of the target sequence (Ex. 2030, p. 24, l. 25 - p. 25, l. 4). For this species, a shared primer pair is not only not required, but cannot be used (id., p. 26, ll. 6-10).

As noted by Wang in its reply (Paper 34, p. 2), "it is possible that other reference sequences described in the '450 specification (e.g., T cell receptor surface sequence or  $\beta$ -actin sequence) are longer and thus have 'substantially more nucleotides' than a target sequence (e.g., where the target viral sequence is the fragment amplified by HIVA and HIVB)."

- ii. **a control sequence comprising a "multi-base insertion" does not require an insertion "preselected" to preserve the primer binding sites of the target sequence and, therefore, does not require or necessarily result in use of a shared primer pair**

33. According to the Murakawa '450 specification,

[a]n additional aid to quantitation of virus levels in patient samples is provided by a reference RNA which can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples.

Such a reference RNA may be a "minigene" or a "maxigene" formed by a multi-base pair insert into or deletion of at least about 20 nucleotides from a unique site. For example a preferred reference RNA includes a 21 base pair insert into the KpnI site of the HIV-1 3' ORF (nef) region of the pGEM92 clone described in Example I. An insert of sequence: ... is appropriate.

The transcription product of this clone is 21 bases longer than the

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authentic HIV-sequence but **still hybridizes to with the 25-mer probe HIVC**. It is therefore distinguishable by size from the authentic viral product.

Such "minigenes" and "maxigenes" not only provide an internal control but also an additional aid to quantitation. Because the quantity of "maxigene" [sic, or] minigene RNA originally included in the amplification reaction is known, the amount of signal obtained from the maxi or minigene amplification product can be related to the signal obtained from the patient sample. Hence, the relative quantitation of the original amount of authentic HIV-1 in the patient sample is provided. [Ex. 2006, p. 6, l. 15 - p. 7, l. 8, emphasis added.]

According to Murakawa, this is the only description in its '450 specification of a control sequence containing "substantially more" nucleotides than the target sequence, i.e., a "maxigene" (Paper 35, p. 19). Further according to Murakawa, since the maxigene "can be amplified and detected by the same oligonucleotides used for the authentic virus RNA samples" and the described site of the example is located between the primer binding sites of the viral RNA and the insertion of the multi-base insert does not destroy the primer binding sites of the control sequence, "a person of skill in the art would have interpreted the 'preselected site' in claim 19 to mean a site selected so as not to disrupt primer binding sites" (id., pp. 21-22). [See also Ex. 1004, ¶ 31, where Dr. Joyce voiced the same opinion.]

First, as noted by Wang in its opposition (Paper 35, p. 9), the term "preselection" never appears in the Murakawa '450 specification. Second, as noted above, the term "preselected" in this context does not have an art-recognized meaning. Third, while a "preferred" RNA control sequence might contain an insertion site that does not disrupt

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primer binding sites in the base (i.e., target) sequence,<sup>14</sup> "it is generally impermissible to limit claim terms to a preferred embodiment or inferences drawn from the description of a preferred embodiment". Bell Atlantic Network Services v. Covad Communications, 262 F.3d 1258, 1273, 59 USPQ2d 1865, 1874 (Fed. Cir. 2001). Thus, as to claim 19, the dispositive question is whether "a reference RNA which can be amplified and detected by the same oligonucleotides used for authentic virus RNA samples" necessarily requires or results in use of a shared primer pair.

34. Dr. Erlich testified that he considered this language "ambiguous" and that he'd "be more likely to interpret oligonucleotides as referring to hybridization probe rather than a primer pair" (Ex 1010, p. 25, l. 25 - p. 28, l. 5).<sup>15</sup>

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<sup>14</sup> Dr. Erlich testified that even if the insert was at a site between two primers, either a shared primer pair or two different primer pairs could be used for amplification (Ex 1010, p. 28, l. 7 - p. 29, l. 3).

<sup>15</sup> On cross-examination, Dr. Erlich testified:

- Q: OK. There's reference to the reference RNA, which can be amplified and decked [sic, detected] by the similar oligonucleotides used for authentic virus RNA samples. Do you see that?
- A: Yes.
- Q: And that's a definition of RNA or a description of a shared primer pair as you've discussed it in your declaration, isn't it?
- A: No, I don't think it is.
- Q: Why not?
- A: For one thing, the language is ambiguous. Nucleotides, the same nucleotides could be interpreted either as the same oligonucleotides probes or conceivably as a shared primer pair. But as it reads here, it's ambiguous.
- Q: But under one interpretation, it can mean a shared primer pair?
- A: As I said, it's ambiguous. And actually the way I would read it, given that the products are distinguishable by size, is that when it says "detected by the same oligonucleotides," I think it's more likely to be a probe than a primer pair.
- Q: Well, it says, "Amplified and detected by the same oligonucleotides."
- A: Yeah. I think in common usage, if you were referring to a, primary you would say amplified by the same nucleotides. And if you meant hybridization, you would say detected by the same nucleotides.
- Q: But it could also be interpreted to mean that they both can be amplified by the same primers and detected by the same oligonucleotides; is that correct?
- A: Well, as I said, it's ambiguous, and it doesn't specify that. There's an additional reason why I thought it was more likely that this phrase detected by the same oligonucleotides

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35. During cross-examination (Ex 2030, p. 54, ll. 3-12), Dr. Joyce testified that

Q: ... So isn't it true that what unique site could be is that it's to make sure that the probe isn't affected rather than that the primer isn't affected, the primers aren't affected?

A: Yeah. That's just, that's not my reading of these detailed descriptions. My reading is it's primer A, primer B, and probe C, all three of which are the same for both the target sequence and the reference sequence. And I drew my Exhibit A that way.

36. That being said, Murakawa '450 Example I describes amplification of an HIV-1 target sequence HIVA and HIVB oligodeoxyribonucleotide (i.e., primer A and primer B) with subsequent hybridization to oligodeoxyribonucleotide probe HIVC (i.e., probe C) (Ex. 2006, pp. 8-9). Example VI is said to be "Example 1 ... repeated with the exception that the primer pair beta actin A and beta actin B is included in the amplification reaction mixture" (*id.*, p. 12). Example VII is said to be "Example 1 ... repeated with the exception that the maxigene primer is included in the reaction mixture" (*id.*, emphasis added).

Thus, Example VII aids in clarifying the ambiguity noted by Dr. Erlich insofar as it provides a basis in Murakawa '450 for construing "preselecting" an insertion site in a control sequence with respect to a target sequence as meaning that the site should be chosen to avoid disrupting probe hybridization. In other words, there is no explicit

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referred to hybridization probes, as there's other language. And I think exhibit perhaps 2005 or -- yeah, I guess it's the previous exhibit, where the language was similar.

It said detected, amplified and detected by the same oligonucleotides. But it referred to a different primer pair.

So if I were to interpret this phrase, using the language of the 2005 exhibit, I'd be more likely to interpret oligonucleotides as referring to hybridization probe rather than primer pair.

Q: But it's a matter of interpretation. You can't say that this language specifically excludes a shared primer pair, can you?

A: It doesn't specify it. It doesn't exclude it.

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disclosure in Murakawa '450 that the "preselected site" should be chosen to avoid disrupting the primer binding sites (although it may be so chosen in a preferred embodiment). Hence, while Murakawa '450 original claims 18, 19 and 30 encompass use of a shared primer pair, they do not require or necessarily result in use of shared primer pair. It is possible to have a maxigene control sequence which can be amplified by different oligonucleotides and detected by the same oligonucleotides used for the target sequence. To find otherwise would be to limit claim terms to a preferred embodiment or inferences drawn from the description of a preferred embodiment. (See Bell Atlantic Network Services, supra, Cir. 1998). "The mere fact that a certain thing may result from a given set of circumstances is not sufficient [to establish inherency]." In re Oelrich, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981) (quoting Hansqirg v. Kemmer, 102 F.2d 212, 214, 40 USPQ 665, 667 (CCPA 1939)). "Inherency ... may not be established by probabilities or possibilities." Continental Can Co. USA v. Monsanto Co., 948 F.2d 1264, 1268-69, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991).

Finally, Murakawa's argument (Paper 35, p. 22) that claim 30, when construed in light of claims 18 and 26 and the Murakawa '450 specification by one skilled in the art, requires a predetermined initial amount of a control sequence that binds the same primer pair as the target sequence is unpersuasive for the reasons given above, i.e., binding to a shared primer pair is neither excluded, required nor a necessary result.

Therefore, none of the earlier Murakawa claims, i.e., Murakawa claims filed or pending as of June 15, 1994 (Exs 1003, 2004-2008, 2021-2022 and 2024-2028), are directed to the same or substantially the same invention as claimed in the Wang 1993

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patent because, although they recite use of a predetermined initial amount of a control sequence, none require or necessarily result in use of shared primer pairs.

**VI. The earlier Murakawa claims cannot be analyzed as a group of related claims to the same invention**

In its opposition (Paper 35, p. 15), Murakawa argues that "all of the Murakawa earlier claims that are drawn to substantially the same invention may be analyzed as a group to determine whether Murakawa has earlier claimed each and every one of the material limitations of the copied claims", citing Corbett for support.

In Corbett, the court held

that there is a substantial difference between that which is to be gathered from the perusal of a group of related claims to the same invention and that which is to be gathered from the perusal of a group of claims to related inventions. The more divergent the subject matter of the individual claims, the less likely it is that the coverage of the interstices therebetween is realized.

Corbett, 568 F.2d at 766, 196 USPQ at 343-44 (original emphasis).

There is no dispute that Murakawa '959 claims 15-17 and 20 require a predetermined initial amount of a control sequence (FF 20). Murakawa still must show that one of the claims in Murakawa '959 contains a shared primer limitation in addition to the predetermined initial amount of control sequence limitation.

Murakawa contends that Murakawa '959 claim 9 uses the same language as Murakawa '450 claim 19, i.e., that the control sequence is "(iii) a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence" (Paper 35, pp. 6-7) and that this language is supported by nearly identical disclosure in each of the Murakawa '959 and

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'450 specifications (Paper 35, pp. 7-8).

37. Murakawa '959 claim 9 reads:

A process as defined by claim 7

[A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood or H-9 cell sample which comprises

- (i) selecting said target viral RNA sequence;
- (ii) simultaneously subjecting (a) said sample and (b) at least one reference nucleotide which does not include said target sequence or which contains substantially more nucleotides than said target sequence;

to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification products produced by step (ii);

(iv) subjecting the denatured amplification products of step (iii) to hybridization conditions separately and sequentially with oligonucleotide probes homologous to said target sequence and to said reference sequence;

whereby hybridization of the probe with the amplified reference sequence provides a positive control and minimizes the possibility of false negative data]

in which said target viral sequence is located within the 3' ORF region of HIV-1 and in which the reference sequence utilized in step (ii) is located in the constant region of the beta chain of the T-cell receptor expressed [sic, in] T-cells affected by HIV-1.

First, we agree that Murakawa '959 claim 9 recites a control sequence which contains substantially more nucleotides than said target sequence, i.e., the constant region of the beta chain of the T-cell receptor. As discussed above, under the conditions of Murakawa '959 claim 9, a shared primer pair could not be used. Second, even assuming arguendo that Murakawa intended to rely on Murakawa '959 claim 7,

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then Murakawa '959 claim 7, for the same reasons as given in regard to Murakawa '450 claim 19, includes, but does not require, a shared primer pair for the same reasons as given above. Third, we note the parallel between Murakawa '450 Examples I, VI and VII and Murakawa '959 Examples I, II and III (Ex. 2005, pp. 5-6). To wit, Murakawa '959 Example III is described as "Example I ... repeated with the exception that the maxigene primer is included in the reaction mixture" (Ex. 2005, p. 7).

Moreover, as noted by Wang in its reply (Paper 34, p. 10), "[a]ll other earlier Murakawa claims are directed to different, albeit related, inventions." A process for minimizing false negative data in the identification of a target viral RNA sequence is not directed to the same invention as the Murakawa claims in interference, i.e., a PCR-based method for determining the amount of a target nucleic acid sequence in a sample by simultaneously amplifying the target nucleic acid sequence and a known amount of an added control sequence with the same oligonucleotide primer pair in a single reaction mixture. In short, neither invention A (reciting only material limitation A) nor invention B (reciting only material limitation B) is the same invention as invention A+B (requiring both material limitations A and B). Thus, none of the earlier Murakawa claims relate to the same or substantially the same invention as claimed in the Wang 1993 patent because none of the Murakawa claims filed or pending as of June 15, 1994 require or necessarily result in both (A) a predetermined initial amount of a control sequence and (b) a shared primer pair.

#### **VII. Conclusion**

Accordingly, involved Murakawa '450 claims 34-35, 38-39 and 42-47 are barred



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DECISION ON PRELIMINARY MOTION

(Murakawa preliminary motion 1)

INTERFERENCE NO. 105,055

DATED APRIL 5, 2004

PAPER NO. 50 IN U.S. SERIAL NO. 07/402,450  
(Paper No. 47 in Interference No. 105,055)

The opinion in support of the decision being  
entered today is not binding precedent of the Board.

Paper 

By: Trial Section Merits Panel  
Board of Patent Appeals and Interferences  
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Filed: 5 April 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES  
(Administrative Patent Judge Carol A. Spiegel)

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ALICE M. WANG, MICHAEL E. DOYLE  
and DAVID F. MARK

Junior Party,  
U.S. Patent 5,219,727  
U.S. Patent 5,476,774

v.

GEORGE MURAKAWA, R. BRUCE WALLACE,  
JOHN A. ZAIA and JOHN J. ROSSI

Senior Party,  
Application 07/402,450

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Patent Interference No. 105,055

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Before: SCHAFFER, TORCZON and SPIEGEL, Administrative Patent Judges  
SPIEGEL, Administrative Patent Judge

DECISION ON PRELIMINARY MOTION  
(Murakawa preliminary motion 1)

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For reasons set forth in the MEMORANDUM OPINION and ORDER (Paper 36) of November 5, 2003, the Board concluded that all of the involved Murakawa claims, i.e., Murakawa claims 34-35, 38-39 and 42-47 are barred under 35 U.S.C. § 135(b)(1) by the 1993 Wang U.S. Patent 5,219,727. Murakawa was ordered to submit one (1) claim that interferes with the claimed subject matter of Wang patents 5,219,727 and 5,476,774 and is not time barred by § 135(b)(1) in order for this interference to continue (Paper 37).

Before us for consideration is Murakawa preliminary motion 1 to add proposed Murakawa claim 50 and to designate it as corresponding to Count 1. We deny Murakawa's motion because its proposed claim 50 does not interfere with the subject matter of the involved Wang claims.

#### **I. Introduction**

This interference concerns a polymerase chain reaction ("PCR") - based method for determining the amount of a target nucleic acid sequence in a sample by simultaneously amplifying the target nucleic acid sequence in a sample and a known amount of an added internal standard sequence ("control sequence") with the same primer pair in a single reaction mixture (Count 1). (See Paper 36, pp. 2-3 for a more complete description of the interfering subject matter.)

Briefly, PCR is an enzymatic DNA amplification method involving repeated cycles of defined steps. The required reagents include a DNA polymerase enzyme, each of

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<sup>1</sup> In this decision, the terms "standard," "reference" and "control" are equivalent when used in reference to the added internal standard nucleic acid. Furthermore, in this decision, the term "predetermined quantity" is equivalent to the term "predetermined amount."

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the four nucleotide building blocks of DNA (i.e., dNTPs A, T, G and C), a source of template DNA containing a target sequence and two oligonucleotide "primers" designed to be complementary to the bases at the 3' ends of the target DNA sequence.

Amplification takes place in three stages, i.e., denaturation, annealing and extension. Denaturation of template DNA separates the double-stranded DNA into two single strands. The primers bind, i.e., hybridize, to their complementary DNA sequences on the single strands during annealing (a large excess of primers is used to ensure that the single strands will bind to the primers instead of each other), resulting in a nucleic acid molecule that is partially double-stranded where the primer is hybridized and partially single stranded where the primer has not hybridized. The polymerase uses the annealed primer as a substrate and sequentially adds a nucleotide to the 3' end of the primer which is complementary to the nucleotide which is "across" from it on the single-stranded portion of the molecule to produce a "primer extension product." Each time this three-step cycle is repeated the products of the previous cycle become new templates for the next cycle such that in each new cycle the amount of the target DNA essentially doubles.

In reverse-transcription PCR, mRNA is transcribed back into cDNA which is then used as the template for PCR.

[See generally, U.S. Patent 4,683,195 issued July 28, 1987 to Mullis et al. (Mullis), entitled "Process for Amplifying, Detecting, and/or Cloning Nucleic Acid Sequences" (Ex 1013); Paper 36, p. 2, n.1.]

According to Wang,

[b]ecause [PCR] amplification is an exponential process, small differences in any of the variables which control the reaction rate, including the length and nucleotide sequence of the primer pairs, can lead to dramatic differences in the yield of PCR product. Analyses which use two sets of unrelated primers, therefore, can only provide a relative comparison of two independent amplification reactions rather than an absolute measure of the mRNA concentration. [Exs 2002 and 2003 at c. 1, ll. 57-65.]

Further according to Wang,

...differences in primer efficiency are difficult parameters to regulate for quantitative analysis. ... As indicated in FIG. 4, the efficiency of amplification by these different primer sets under the same PCR amplification conditions varied over a range of several orders of magnitude. For instance, the IL-1 $\beta$  primers are 10<sup>3</sup>-fold more efficient than the apo-E primers. Thus, it is critical to use the same primers for amplification of both the target mRNA and the internal standard in any attempts to quantitate mRNA expression by PCR. [Exs 2002 and 2003 at c. 16, l. 62 - c. 17, l. 8.]

Here, Wang '727 claim 1 is not anticipated by proposed Murakawa claim 50 because proposed claim 50 does not explicitly or inherently require use of the same primer pair to amplify both target and reference nucleic acid sequences as required by Wang '727 claim 1. Further, Wang '727 claim 1 is not rendered obvious by proposed Murakawa claim 50 in view of certain prior art because modifying proposed claim 50 as suggested would not have resulted in Wang's claimed invention.

## **II. Findings of fact (FF)**

The following findings of fact are supported by a preponderance of the evidence.

1. The junior party is ALICE M. WANG, MICHAEL D. DOYLE and DAVID F. MARK (Wang).
2. Wang is involved in the interference on the basis of two patents:
  - (i) U.S. Patent 5,219,727 ("the Wang 1993 patent"), which issued June 15,

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1993, based on application 07/413,623, filed September 28, 1989, and

- (ii) U.S. Patent 5,476,774 ("the Wang 1995 patent"), which issued December 19, 1995, based on application 08/028,464, filed March 9, 1993.

3. The Wang 1993 patent has been accorded benefit for the purpose of priority of U.S. application 07/396,986, filed August 21, 1989. The Wang 1995 patent has been accorded benefit for the purpose of priority of both U.S. application 07/413,623 (now the Wang 1993 patent) and U.S. application 07/396,986.

4. Both the 1993 and 1995 Wang patents are assigned to Roche Molecular Systems, Inc.

5. The senior party is GEORGE J. MURAKAWA, R. BRUCE WALLACE, JOHN A. ZAIA and JOHN J. ROSSI (**Murakawa**).

6. Murakawa is involved in the interference on the basis of application 07/402,450 ("Murakawa '450"), filed September 1, 1989.

7. Murakawa '450 has been accorded benefit for the purpose of priority of

- (i) U.S. application 07/148,959, filed January 27, 1988 ("Zaia '959"), and
- (ii) U.S. application 07/143,045, filed January 12, 1988 ("Murakawa '045").

8. Murakawa '450 is assigned to the City of Hope.

9. There are two counts in the interference. Count 1 is defined by Wang (5,219,727) claim 1 or any of Wang (5,476,774) claims 5, 15 or 17 or any of Murakawa (07/402,450) claims 34, 35, 44, 46 or 47. Count 2 is defined by Wang (5,476,774) claim 1 or Murakawa (07/402,450) claim 45. [Paper 1, p. 5.]

10. The claims of the parties are:

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Wang (5,219,727)	1-10
Wang (5,476,774)	1-18
Murakawa (07/402,450)	34-35, 38-39, 42-47

11. The claims of the parties which correspond to Count 1 are:

Wang (5,219,727)	1-4, 6-10
Wang (5,476,774)	5-7, 10-12, 15-18
Murakawa (07/402,450)	34-35, 38-39, 42-44, 46-47

12. The claims of the parties which correspond to Count 2 are:

Wang (5,219,727)	none
Wang (5,476,774)	1-3, 8-9
Murakawa (07/402,450)	45

13. The claims of the parties which do not correspond to either Count 1 or Count 2, and therefore are not involved in the interference, are:

Wang (5,219,727)	5
Wang (5,476,774)	4, 13-14
Murakawa (07/402,450)	none

14. Murakawa claims 34-35, 38-39 and 42-47 are barred under 35 U.S.C.

§ 135(b)(1) by the 1993 Wang patent 5,219,727 (Paper 36).

15. Murakawa was ordered to submit one (1) claim that interferes with the claimed subject matter of the 1993 and 1995 Wang patents (Wang '727 and Wang '774) and (2) is not time barred by § 135(b)(1) in order for this interference to continue (Paper 37).

Other findings of fact follow below.

## II. Murakawa preliminary motion 1

Pursuant to the Order dated November 5, 2003 (Paper 37) and further pursuant to 37 CFR §§ 1.633(c)(2) and 1.627(c)(2), Murakawa moves to add proposed claim 50 to the involved Murakawa '450 application and to designate this claim as corresponding

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to Count 1 (Paper 38). Wang opposes (Paper 44); Murakawa replies (Paper 45).

**A. There is no interference-in-fact between the subject matter of proposed Murakawa '450 claim 50 and the subject matter of Wang '727 patent claim 1**

In order for there to be an interference-in-fact between claims, two way anticipation or obviousness must be established. An interference-in-fact exists when at least one claim of a party that is designated to correspond to a count and at least one claim of an opponent that is designated to correspond to the count define the same patentable subject matter. 37 CFR § 1.601(j). Invention A is the same patentable invention as an invention B when invention A is the same as (35 U.S.C. 102) or is obvious (35 U.S.C. 103) in view of invention B assuming invention B is prior art with respect to invention A. 37 CFR § 1.601(n). A patentable distinction in either direction requires a finding of no interference-in-fact. See Winter v. Fujita, 53 USPQ2d 1234 (BPAI 1999); Eli Lilly & Co. v. Board of Regents of the University of Washington, 334 F.3d 1264, 67 USPQ2d 1161 (Fed. Cir. 2003).

**1. comparison of proposed Murakawa claim 50 and Wang '727 claim 1**

16. The following chart compares the language of Wang '727 claim 1 and Murakawa proposed claim 50 (emphasis added).

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Proposed Murakawa claim 50		Wang '727 claim 1
50. A process for quantitation of		1. A method for quantifying
<b>a target viral RNA sequence</b>		<b>a target nucleic acid segment</b>
in a peripheral blood cell sample which comprises		in a sample, which method comprises the steps of:
(i) selecting said target viral RNA sequence;	(a) adding to said sample	
	(ii) simultaneously subjecting	
	(a) said sample and	a predetermined initial amount of standard nucleic acid segment wherein said <b>standard nucleic acid segment binds to same primers as are bound by said target nucleic acid segment</b> in a reaction mixture;
	(b) a predetermined quantity of at least one <b>synthetic RNA sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence</b>	
to polymerase chain reaction amplification under conditions to simultaneously amplify said target sequence if present in said sample and said reference sequence;		(b) treating said sample under conditions suitable for carrying out a polymerase chain reaction, wherein said nucleic acids are rendered single-stranded and exposed to an agent for polymerization, deoxynucleoside 5' triphosphates, and a pair of oligonucleotide primers, wherein said <b>pair of primers is specific for both the target and standard nucleic acid segments</b> , such that an extension product of each primer of said pair can be synthesized using separate strands of the target and standard segments as a template for synthesis, such that the extension product of one primer, when it is separated from the template strand, can serve as a template for the synthesis of the extension product of the other primer of said pair <b>wherein said amplified target and standard segments are distinguishable by size or by the use of internal probes</b> , wherein said internal probes may be differentially labeled for each of said amplified target and standard segments;
(iii) denaturing the amplification products produced by step (ii);		(c) separating the primer extension product from the templates on which they were synthesized to form single-stranded molecules; (d) repeating steps (b) and (c) on the single stranded molecules produced in step (c) at least once, whereby each repeat of steps (b) and (c) is one amplification cycle;

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<p>(iv) subjecting said denatured amplification products of step (iii) to hybridization conditions separately and sequentially with labeled probes homologous to said target sequence and to said reference sequence and detecting the presence or absence of the target sequence and the presence of the reference sequence in the amplification products of step (iii) by Southern blot hybridization with said labeled probes,                  each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subsection of said amplification products to hybridization with another of said probes;</p>	
<p>(v) determining whether said amplified target and reference sequences hybridized with said probes homologous therewith; and                  (vi) determining the relative quantification of the target sequence by comparison with the amount of signal obtained from the hybridized target sequence with the amount of signal obtained from the hybridized predetermined quantity of the reference sequence.</p>	<p>(e) measuring the amounts of the amplified target and standard segments produced in step (d); and                  (f) calculating from the amplified target and standard segments produced in step (d) the amount of said target nucleic acid segment present in the sample before amplification.</p>

17. At the outset, it is noted that proposed Murakawa claim 50 and Wang '727 claim 1 present a mix of genus and species elements. Wang '727 claim 1 is generic as to the type of target nucleic acid sequence and sample, whereas proposed Murakawa claim 50 is specific to a viral RNA target sequence and a peripheral blood sample. The reference sequence of proposed Murakawa claim 50 is a synthetic RNA sequence selected from one of two genera, i.e., a reference sequence which does not include the target sequence or a reference sequence which includes substantially more nucleotides than the target sequence. The reference sequence of Wang '727 claim 1 is a species,

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i.e., a nucleic acid sequence which is expressly required to bind to the same PCR primers as the target sequence. Both proposed Murakawa claim 50 and Wang '727 claim 1 expressly encompass reference sequences which are distinguishable by size from the target sequence, but only Wang '727 claim 1 requires the reference sequence to bind to the same primer pair as the target sequence. In addition, proposed Murakawa claim 50 detects the presence or absence of PCR amplification products by a specific method, i.e., by a Southern blot hybridization assay, whereas Wang '727 claim 1 is not so limited. Finally, proposed Murakawa claim 50 determines the relative quantity of target sequence present in the sample before amplification by comparing the amount of signal obtained from the target and reference sequences. Wang '727 claim 1, on the other hand, calculates the actual amount of target sequence present in the sample before amplification.

18. According to Wang,

[t]he amount of the target nucleic acid segment present in the sample prior to amplification is determined using a standard curve. The standard curve is generated by plotting the amount of the standard segment produced in a polymerase chain reaction against varying, but known, amounts of the RNA present before amplification. For accuracy, the amount of standard segment present before amplification is varied by serial dilution of the co-amplification reaction mix. The amount of target segment produced in the polymerase chain reaction is then compared to the standard curve to determine the amount of target segment present in the sample prior to amplification. Alternatively, the standard curve may be generated by plotting the amount of standard and target segments produced against the number of amplification cycles. To ensure accuracy, it is preferred that the number of amplification cycles is varied by removing aliquots from one co-amplification reaction mixture after different numbers of amplification cycles have been completed. [Exs 2002 and 2003 at c. 4, ll. 14-33.]

19. Wang's invention is said to be

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...far superior to determinations of the amount of a nucleic acid segment in a sample as a relative, rather than absolute, amount. Further, the method is [said to be] far more accurate than when an absolute amount is derived by employing a second set of oligonucleotide primers to amplify the standard, wherein that set of primers is different from the set used to amplify the target segment. [Exs 2002 and 2003 at c. 4, ll. 34-41.]

20. Murakawa '450 Example I describes amplifying an HIV-1 target sequence with primer pair HIVA and HIVB with subsequent Southern blot detection of primer extension products by hybridization to a radiolabelled probe HIVC (Ex 2006, pp. 8-9). Example VI is said to be "Example I ... repeated with the exception that primer pair actin A and beta actin B is included in the reaction mixture" (*id.*, p. 12). Example VII is said to be "Example I ... repeated with the exception that the maxigene [i.e., reference sequence] primer is included in the reaction mixture" (*id.*).

Both generic reference sequence examples, i.e., a reference sequence which does not include the target sequence or a reference sequence which includes substantially more nucleotides than the target sequence (a "maxigene"), in Murakawa '450 are amplified using a different pair than that used to amplify the target sequence.

21. According to Murakawa '450, its reference sequence provides an

... aid to quantitation. Because the quantity of "maxigene" [or] minigene RNA originally included in the amplification mixture is known, the amount of signal obtained from the maxi or minigene amplification product can be related to the signal obtained from the patient sample. Hence, the relative quantitation of the original amount of authentic HIV-1 in the patient sample is provided. [Ex 2006, p. 7, ll. 1-8.]

Thus, we understand proposed Murakawa claim 50 to determine the "relative" amount target sequence in the original sample by taking an aliquot of the PCR amplification mixture at some point, performing a Southern blot analysis using labelled

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probes to detect the presence or absence of the target sequence and the presence of the reference sequence, and comparing the signal obtained from labels hybridized (i.e., bound) to target and reference primer extension products to determine the "relative" amount of target sequence. To wit, the aliquot is applied to a gel and then electrophoresed to separate target and reference primer extension products by size; the separated products are transferred to a membrane; and, labelled probes are separately and sequentially reacted with the transferred products. Presence of the reference sequence is indicated by a signal produced by the label of the probe hybridized to the transferred reference primer extension product. Presence of the target sequence is indicated by a signal produced by the label of the probe hybridized to any transferred target primer extension product. Absence of target signal may indicate no, or an undetectable amount, of target sequence in the original sample in cases where a reference signal is detected; or, may indicate a false negative result in cases where no reference signal is detected. The relative amount of target sequence in the original sample is determined by comparing the amount of signal obtained from the label hybridized to target primer extension product vis-a-vis the amount of signal obtained from the label hybridized to reference primer extension product, i.e., a determination based on a ratio -- a de facto "single point standard curve" -- in contrast to the multi-point standard curve inherent in Wang '727 claim 1. It is uncertain whether the target and reference amplification reactions of proposed Murakawa claim 50 are proceeding equally, e.g., both proceeding with the same efficiency at the same rate. In other words, the method of proposed Murakawa claim 50 is semi-quantitative at best,

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whereas the method of Wang '727 claim 1 is quantitative.

**2. step (iv) of proposed Murakawa claim 50 is anticipated by Wang '727 claim 1**

A single prior art reference anticipates a patent claim if it expressly or inherently describes each and every limitation set forth in the patent claim. Verdegaal Bros., Inc. v. Union Oil Co., 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). Inherent anticipation requires that the missing descriptive material is "necessarily present," not merely probably or possibly present, in the prior art. In re Robertson, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citing Continental Can Co. USA, Inc. v. Monsanto Co., 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991)). However, "the disclosure of a chemical genus . . . constitute[s] a description of a specific compound" within the meaning of section 102(b) where the specific compound falls within the ambit of a "very limited number of compounds." In re Schaumann, 572 F.2d 312, 315, 316, 197 USPQ 5, 8 (CCPA 1978).

Wang contends that proposed Murakawa claim 50 is not anticipated by Wang '727 claim 1 because Wang '727 claim 1 does not contain step (iv) of proposed claim 50 (Paper 44, pp. 16-17). Wang further contends that such a step is neither recited, taught nor "inherently described" in any other involved Wang claim or patent (*id.*, p. 17).

Murakawa responds that Wang '727 claim 1 contains a generic recitation of proposed claim 50 step (iv), i.e., Wang recites a genus which anticipates the species of Murakawa (Paper 45, p. 11). According to Murakawa, since the use of probes and the detection of hybridized probes, e.g., Southern blot hybridization, were in the public's possession as shown by Mullis (Ex 1013), Wang '727 claim 1 contains all of the

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limitations of proposed claim 50 (id.). Murakawa relies on In re Donahue, 766 F.2d 531, 534, 226 USPQ 619, 621-22 (Fed. Cir. 1985) and Brown v. 3M, 265 F.3d 1349, 1351, 60 USPQ2d 1375, 1376 (Fed. Cir. 2001), cert. denied, 122 S. Ct. 1436 (2002) to support its argument.

Here, we agree that Southern blot analysis is such an old and well-known means for detecting DNA of different sizes in a sample that one of ordinary skill in the art would have immediately envisaged using Southern blot analysis to measure PCR amplification products of different sizes regardless of the size of the genus of methods for measuring PCR amplification products generically recited in Wang '727 claim 1. In re Petering, 301 F.2d 676, 682, 133 USPQ 275, 280 (CCPA 1962). Indeed, the Wang '727 specification itself exemplifies determining the amount of amplified product by electrophoresis and visualization of the amplified product by hybridization with a labeled probe, e.g., by extrapolation from an autoradiograph (Ex 2002, c. 11, l. 66 - c. 12, l. 4).

Technically, Murakawa's reply fails to comply with STANDING ORDER § 26(c)(3)<sup>2</sup>, i.e., Murakawa has not provided a specific citation<sup>3</sup> by column and line to

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<sup>2</sup> STANDING ORDER § 26(c)(3) reads: "In presenting a reply, a party shall set out in the following order: ... (3) Any additional facts upon which the moving party intends to rely to rebut additional facts alleged by the opposing party with a citation to the evidence and an explanation as to why each additional fact was not set out in the motion."

<sup>3</sup> As discussed in STANDING ORDER § 26(a), "Citation to the evidence must be specific, i.e., (1) by column and line of a patent, (2) page, column and paragraph of a journal article and (3) page and line of a cross-examination deposition transcript. Citations to an entire document or numerous pages of a cross-examination deposition transcript do not comply with the requirement for a citation to the record. In this respect, the Trial Section adopts as its policy the rationale of Cintec Nutrition Co. v. Baxa Corp., 44 USPQ2d 1719, 1723 n.16 (N.D. Ill. 1997), which notes that where a party points the court to multi-page exhibits without citing a specific portion or page, the court will not pour over the documents to extract the relevant information, citing United States v. Dunkel, 927 F.2d 955, 956 (7th Cir. 1991). Nor will the board take on the role of an advocate for one of the parties. Compare Ernst Haas Studio, Inc. v. Palm Press, Inc., 164 F.3d 110, 111-12, 49 USPQ2d 1377, 1378-79 (2d Cir. 1999)."

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where Mullis (Ex 1013) describes measuring amounts of amplified target and reference sequences by Southern blot hybridization. Mullis (Ex 1013) comprises twenty-six (26) claims, twelve (12) figures and forty (40) columns of disclosure. It is not the function of the Board to search through a record to find evidence supporting a party's position. However, detection of size-distinguishable DNA in a sample using Southern blot analysis was so well known that we decline to dismiss Murakawa's reply for purely technical reasons. However, Murakawa is cautioned to follow the procedural rules set forth in the STANDING ORDER or bear the consequences of failing to do so in the future.

Although Murakawa has argued this as "anticipation," it has also pointed to a difference which makes obviousness the more applicable standard. Wang urges that hold this mislabeling to be dispositive. On the facts of this case we decline to do so. Wang had notice of the substance of Murakawa's argument and thus is not prejudiced. In the final analysis, the question is whether we, on the Director's behalf, are of the opinion that the claims interfere. Whether the analysis is called anticipation or obviousness, we see no patentable distinction on this point.

Based on the above, we find that step (iv) of proposed Murakawa claim 50 is anticipated by Wang '727 claim 1.

3. **Wang '727 claim 1 is not anticipated by proposed Murakawa claim 50 because proposed claim 50 (a) does not explicitly or inherently require use of a shared primer pair to amplify both target and reference sequences as required by Wang claim 1 or (b) recite a sufficiently small genus such that one of ordinary skill in the art would immediately envisage use of a shared primer pair to amplify both target and reference sequences as required by Wang claim 1**

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Murakawa argues that

[p]roposed claim 50 has all the elements of claim 1 of the Wang '727 patent except that proposed claim 50 specifies a genus of the reference sequence whereas claim 1 of the Wang '727 patent specifies only a species of the reference sequence, namely, a reference sequence which uses the same primer pair as the target nucleic acid. The genus of proposed claim 50 encompasses the species of claim 1 of the Wang '727 patent. ... The genus of proposed claim 50 includes four species, namely, (i) a synthetic RNA species which does not include the target sequence ..., (ii) a synthetic reference sequence which includes substantially more nucleotides than the target sequence ... and (iii) a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA with respect to said target sequence (Fact 24), which also includes (iv) a sequence which uses the same primer pair as the target sequence for amplification .... The reference sequence of proposed claim 50 is a small genus which, therefore, anticipates the reference sequence species of claim 1 of the Wang '727 patent. In re Petering, 301 F.2d at 682; Bristol-Myers, 246 F.3d at 1380; In re Schaumann, 572 F.2d at 316-317. As described above, proposed claim 50 also contains the elements of (i) the simultaneous amplification of a sample and predetermined quantity of the reference sequence and (ii) determining the quantity of target nucleic acid present in the sample by comparing the amounts of amplified target nucleic acid and the amount of amplified predetermined quantity of reference sequence. Thus, proposed claim 50 contains all of the elements of claim 1 of the Wang '727 patent, and thereby anticipates claim 1 of the Wang '727 patent, assuming that proposed claim 50 is prior art. PPG Indus., 75 F.3d at 1565. [Paper 38, ¶ bridging pp. 19-20, citations to Murakawa's "STATEMENT OF MATERIAL FACTS SUPPORTING THE MOTION" omitted.]

Furthermore, according to Murakawa, "[p]roposed claim 50 is claim 27 as originally filed in Murakawa's involved application written in independent form incorporating all of the limitations of the claims from which it depends, i.e., claims 26 and 18" (*id.*, p. 1).

In rebuttal, Wang presents three arguments. First, Wang contends that "the Board has already ruled that [original] claim 27 [i.e., proposed claim 50] does not contain every limitation of, and thus cannot anticipate, Wang claim 1" (Paper 44, p. 19).

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In the Decision on Wang Preliminary Motion 1 (Paper No. 36), at p. 22, the Board held that "none of the earlier Murakawa claims, i.e., Murakawa claims filed or pending as of June 15, 1994 ... are directed to the same or substantially the same invention as claimed in the Wang 1993 patent because ... none require or necessarily result in use of shared primer pairs." The "earlier Murakawa claims" include Murakawa claim 27. Paper No. 36 at 13, n.11. The "invention claimed in the Wang 1993 patent" includes the invention of Wang claim 1. Thus, the Board has already ruled that claim 27 differs in a material limitation from Wang claim 1. However, anticipation requires that every limitation of a claim can be found, either expressly or inherently, in a prior art reference. See e.g., Verdegaaal Bros., 814 F.2d at 631, 2 USPQ2d at 1053. Consequently, the Board has already ruled that claim 27 does not contain every limitation of, and thus cannot anticipate, Wang claim 1. [Paper 44, ¶ bridging pp. 18-19.]

Therefore, Wang contends, again citing to the Decision on Wang Preliminary Motion 1 (Paper 36, p. 22, "binding to a shared primer pair is neither excluded, required nor a necessary result"), that Murakawa should not be allowed to construct a genus that explicitly recites shared primer pairs (Paper 44, ¶ bridging pp. 19-20). In reply, Murakawa argues that its "[p]roposed claim 50 encompasses a genus of reference sequences, including the species of a reference sequence that uses a shared primer pair with the target, as recognized by the Board" (Paper 45, p. 12).

The question joined by Wang and Murakawa is whether one skilled in the art, given the genus of reference sequences recited in proposed Murakawa claim 50, would "at once envisage" the species of Wang '727 claim 1, i.e., a reference sequence which binds to the same primers as are bound by the target sequence in a PCR amplification reaction. Petering, 301 F.2d at 682, 133 USPQ at 280.

Wang's second argument answers the above question in the negative. Wang points out that the process of proposed Murakawa claim 50 uses "at least one synthetic

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RNA sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence" (Paper 44, p. 20).<sup>4</sup>

According to Wang, the recited synthetic RNA sequence "can be any synthetic RNA sequence, of any size, limited only in that it does not include the target sequence" or a "synthetic RNA sequence ... which includes substantially more nucleotides than said target sequence" ... which is limited, not by sequence, but by size" (*id.*). In either case, Wang argues that the number of possible sequences encompassed thereby constitutes an infinitely large genus (*id.*). Wang further argues that the deposition testimony of Dr. Joyce (Ex 2030 at pp. 23:16-27:25), Murakawa's expert witness, "clearly suggests that there are uncalculated species within the genus of claim 18 (and so within the genus of proposed claim 50)" (*id.*).

22. Dr. Gerald F. Joyce testified (Ex 2030 at pp. 23:16-27:25)

- Q. Let's look at Paragraph 13. In this paragraph you point to this specific passage that you quote from Claim 18 about, synthetic RNA sequence which does not include the target sequence or which includes substantially more nucleotides than said target sequence.
- A. Yes.
- Q. Now, that phrase includes numerous possibilities, doesn't it?
- A. Yes, it does.
- Q. One possibility is that you have a reference sequence that's the same length as the target sequence but does not include the target sequence, right?
- A. That's a possibility.
- Q. And for that possibility you cannot use shared primer pairs, right?

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<sup>4</sup> The Decision on Wang Preliminary Motion 1 noted that

(i) the testimony of two expert witnesses, i.e., Drs. Joyce and Ehrlich, agreed that the term "substantially more", in the context of Murakawa '450 claims 18, 19 and 30, did not have an ordinary and customary meaning to one skilled in the art (Paper 36, p. 17) and

(ii) a control sequence that contains "substantially more" nucleotides than a target sequence, but does not contain any of the target sequence, could not use a shared primer pair (*id.*, p. 18).

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- A. For that possibility you could not use shared primer pairs, correct.
- Q. Isn't it also true that you'd need to use at least two probes to detect and distinguish the target from the reference under that possibility?
- A. Not necessarily. It would be possible to place a target sequence for the probe within the reference sequence and have no other aspect of the reference sequence be the target sequence. In other words, you could have an exogenous probe sequence in both a target and a reference that isn't part of the natural target sequence. I'm just, it just is a possibility.
- Q. Okay. So you could have an insert into a reference sequence that contained none of the target sequence?
- A. Correct.
- Q. And then have that insert be the target for the probe?
- A. That could be done.
- Q. Okay. A second possibility is that you could have a reference sequence that contains substantially more nucleotides than the target sequence but does not contain the target sequence, right?
- A. Yes, that's another possibility.
- Q. And with this second possibility with the exception that you gave before you'd need at least two probes to test for false negatives, right?
- A. All right, let's get the facts here straight. Put the whole thing together again. I want to answer correctly here.
- Q. Sure. That's fine. Now, a second possibility we're talking about where the reference sequence contains substantially more nucleotides than the target sequence.
- A. Right.
- Q. For that you would need to have two probes to test for false negatives unless you made the insertion for the probe that you talked about early?
- A. You said it slightly different the second time. I don't want to put words in your mouth. But I think what you mean is for that circumstance where the reference contains substantially more nucleotides than the target sequence and doesn't contain any of the target sequence.
- Q. That's right.
- A. And excluding the possibility of inserting a probe hybridization sequence within both the target and reference sequence would you then require separate primer pairs; is that the question?
- Q. Yes.
- A. And the answer is, yes, that's possible.
- Q. And it's also true for this second possibility that you cannot use shared primers, right?
- A. It's possible for this second possibility contingent on

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excluding the insertion of the probe sequence that you could not use shared primers. You can see why I had trouble with this, too. There's many possibilities allowed within this claim.

Q. Let me, let's talk about a third possibility. A third possibility is you have a reference sequence like the second one we talked about, except that it contains substantially fewer nucleotides than the target sequence, right?

A. That is a possible construction.

Q. And for that possibility you cannot use shared primers, right?

A. If there's, again, with the restriction there's no exogenous insertion of hybridization sites beyond the starting sequences. So with that caveat on top of that possibility, yes, that's another way in which you could in principle operate without shared primer pairs.

Q. And the answer you gave about needing two probes for the first and second possibilities, the same answer would apply to the third possibility?

A. It could apply to the third possibility as well.

Q. Now, I'm going to talk about something that you mentioned before which is I think there are fourth, fifth, and sixth possibilities.

A. At least.

Q. Which are the same as the first three except the reference sequence contains part of the target sequence, right?

A. Yes.

Q. And all of those fourth, fifth, and sixth possibilities can be practiced without the use of shared primer pairs, right?

A. Optionally they could be practiced without the use of shared primer pairs, yes. Not necessarily, but optionally.

Q. If the portion of the target sequence used in the reference sequence did not include the portion to which one of the primers of the target sequence binds, then it would not be used, possible to use shared primer pairs, right?

A. Excluding the possibility of exogenous placement of hybridization sites for primers and/or probes, yes.

In reply, Murakawa focuses its response on the second of the two Markush members recited in its proposed claim 50, i.e., a synthetic RNA sequence which includes substantially more nucleotides than the target sequence (Paper 45, pp. 12-13). Murakawa argues that "the only difference between the size differentiating reference sequence claim 1 of the Wang '727 patent [see step (b), "wherein said amplified target

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and standard segments are distinguishable by size"] and the reference sequence of proposed claim 50 which differs from target sequence by containing substantially more nucleotides is that claim 1 of the Wang '727 patent uses a shared primer pair with the target sequence" (Paper 45, p. 13).

Use of a shared primer pair is precisely the limitation at issue. It is not a matter of size so much as sequence. Use of a shared primer pair requires that the target and reference sequences share a common nucleotide sequence (see e.g., Paper 36, p. 2, n.1, primers are designed to be complementary to the bases at the 3' ends of a target DNA sequence).

Assuming arguendo that reference sequences distinguishable by size from target sequences constitute a defined, albeit very large, genus, the question is still whether one skilled in the art would "at once envisage" reference sequences having 3' end nucleotide sequences in common with a target sequence, i.e., reference sequences which bind to the same primers as the target sequence in the same PCR amplification reaction mixture.

In view of this record, including the testimony of Dr. Joyce cited above, we find that proposed Murakawa claim 50 would not have immediately suggested using a reference sequence which binds to the same primers as the target sequence in the same PCR amplification reaction mixture. The definition of a reference sequence distinguishable from a target sequence by size is broad and general and does not particularly suggest a reference sequence which binds to the same primer pair as a target sequence. For example, the Murakawa '450 specification suggests using a

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reference sequence which is "present in the expression products of all or virtually all of the cells of a ... sample" (Ex 2006, p. 4). An "ubiquitous" reference sequence may be size-distinguishable and sequence-distinguishable from a target sequence, which may or may not be present in a sample. Thus, regardless of whether a size-distinguishable class of reference sequences encompasses a vast, perhaps infinite number of reference sequences, Murakawa has not pointed us to an explicit or implicit suggestion of a more limited class of reference sequences which binds to the same primers as the target sequence in the same PCR amplification reaction mixture as recited in Wang '727 claim 1.

While Dr. Joyce has testified to the possibility of this limited class within the broad class of size-distinguishable reference sequences, the issue is whether proposed Murakawa claim 50 identifies reference sequences that bind to the same primer pairs as are bound to a target sequence in a PCR reaction with sufficient specificity to constitute a description thereof within the purview of 35 U.S.C. § 102. On this record, we think not. As noted by Wang in its opposition (Paper 44, ¶¶ bridging pp. 20-21), the rationale of Petering, Schaumann and Bristol-Myers is not applicable to the facts of this case. The "prior art genus" of proposed claim 50 does not consist of a few species with a common structure such that one of ordinary skill in the art would "at once envisage each member" of the encompassed species.

Finally, Wang argues that

it should be noted that the reference sequence of Murakawa proposed claim 50 is limited to viral RNA, while claim 1 of the Wang '727 patent recites "nucleic acids." In this aspect, the Wang claim is broader than Murakawa proposed claim 50. Claim 8 of the Wang '727 patent, which

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depends indirectly from claim 1, explicitly relates to quantitation of HIV proteins, among others. However, Murakawa does not argue that its claims are directed to the same patentable invention as claim 8 of the Wang '727 patent, and pursuant to Section 13.7 of the Standing Order, Murakawa should not be heard to do so in its reply. [Paper 44, p. 21.]

Except for Murakawa's general response noted above, i.e., the only difference between the reference sequences of Wang '727 claim 1 and proposed Murakawa claim 50 is that the reference sequence of Wang '727 claim 1 uses a shared primer pair with the target sequence, no more specific response to Wang's final argument is apparent in Murakawa's reply (Paper 45, pp. 12-13).

Based on the above, we find that Wang '727 claim 1 is not anticipated by proposed Murakawa claim 50, assuming proposed claim 50 to be prior art with respect to Wang '727 claim 1.

**4. combining proposed Murakawa claim 50 with the 1988 Murakawa article (Ex 1012) and the Cantin '802 patent (Ex 1020) does not lead to the invention of Wang '727 claim 1.**

First, as noted by Murakawa in its reply (Paper 45, p. 14), obviousness is determined "at the time the invention was made." 35 U.S.C. § 103(a). Assuming arguendo that Wang '727 claim 1 is entitled to its earliest possible effective filing date of August 21, 1989, then both the 1988 Murakawa article (Ex 1012)<sup>5</sup> and the Cantin '802 patent<sup>6</sup> qualify as prior art against Wang '727 claim 1.

Second, as moving party, Murakawa has the burden of establishing by a

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<sup>5</sup> Murakawa et al., "LABORATORY METHODS: Direct Detection of HIV-1 RNA from AIDS and ARC Patient Samples," *QNA*, Vol. 7, No. 4, pp. 287-295 (1988) (Ex 1012, "the 1988 Murakawa article").

<sup>6</sup> U.S. Patent 5,110,802 issued May 5, 1992 to Cantin et al. (Cantin), entitled "Oligonucleotide Phosphonates and Method of Inhibiting a Human Immunodeficiency Virus in vitro Utilizing Said Oligonucleotide Phosphonates" (Ex 1020). Cantin is based on application 07/073,189, filed July 14, 1987.

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preponderance of the evidence, that the invention of Wang '727 claim 1 would have been obvious over proposed Murakawa claim 50 in view of the 1988 Murakawa article and the Cantin '802 patent.

According to Murakawa, its proposed claim 50 differs in failing to recite a reference sequence which is PCR amplified simultaneously with the target sequence using the same primer pair as required by Wang '727 claim 1. Further according to Murakawa, the 1988 Murakawa article discloses (i) a reference sequence that is a target sequence with a multi-base insertion and (ii) use of a shared primer pair to amplify target and reference sequences simultaneously **for confirmation of negative results** (Paper 38, pp. 10-11, asserted facts 29 and 30). Murakawa directs our attention to Ex 1012, "page 292, right column above Fig. 7, Fig. 7 legend and Fig. 8 legend" and "page 293, first full paragraph" (*id.*). Still further according to Murakawa,

[iii] [t]he Cantin '802 patent discloses the simultaneous PCR amplification of a target viral RNA sequence and a fixed concentration of a reference sequence with an insert between the primer sites of the target sequence. MX 1020 at column 4, lines 45-53. [and]

[iv] ... that the initial amount of reference sequence template remains constant thereby enabling one **to determine the ratio of amplification of the reference sequence template versus the sample template before and after treatment to determine the effect of treatment on viral RNA synthesis**. MX 1020 at column 4, lines 57-60 and lines 44-45. [Paper 38, p. 14, asserted facts 48 and 49, emphasis added.]

Thus, Murakawa contends that "[i]t would have been obvious to use the reference sequence of Murakawa et al. (MX 1012) in the process for the quantitation of a target viral RNA sequence of proposed claim 50 in light of the Cantin '802 patent (MX 1020) with a reasonable likelihood of success" (Paper 38, ¶ bridging pp. 20-21).

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Assuming arguendo that it would have been obvious to use the reference sequence of the 1988 Murakawa article (Ex 1012) as the synthetic RNA reference sequence in the method of proposed Murakawa claim 50 in light of the Cantin' 802 patent (Ex 1020), such a combination would not lead to the invention of Wang '727 claim 1.

First, although the preamble of proposed claim 50 recites a "process for quantitation of a target viral RNA sequence," proposed claim 50 does *not* contain the step of calculating the [absolute] amount of target RNA in the sample that is found in claim 1 of the Wang '727 patent" (Wang's opposition, Paper 44, p. 22). Instead, proposed claim 50 recites "determining the **relative quantification** of the target sequence" (step (v), emphasis added).

Second, the 1988 Murakawa article discusses direct detection of HIV-1 nucleic acid sequences (i.e., without a prior culturing step) by a two-step technique -- viral RNA is transcribed back into its complementary DNA followed by PCR amplification of the transcribed DNA (i.e., a reverse-transcription PCR). According to the 1988 Murakawa article, a prokaryotic T7 RNA polymerase promoter sequence was appended to one of the priming oligonucleotides (i.e., HTLVAT7) to enhance the efficiency of the PCR technique (Ex 1012, abstract). HTLVAT7 adds 22 bases constituting the promoter recognition sequence for T7 RNA polymerase to primer HTLVA (*id.*, p. 288, c. 2, second sentence, last paragraph). Figure 7 of the 1988 Murakawa article illustrates PCR amplification of known amounts of RNA, i.e., 0.1 ng prepared from target pGM92 (containing the 3' open reading frame of HIV) and reference pGM92+21 sequences,

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obtained using an HTLVAT7 oligonucleotide in place of the HTLVA oligonucleotide primer and suggests "a significant amplification by the transcription step" using T7 RNA polymerase (Ex 1012, p. 292, Fig. 7 legend, and paragraph bridging pp. 292-293).

Assuming arguendo that pGM92+21 is a reference sequence according to proposed claim 50 (i.e., a synthetic RNA sequence which includes substantially more nucleotides than said target sequence), the 1988 Murakawa article discusses making such

an internal control within our PCR reactions by constructing a plasmid with a small insertion in the targeted region **to determine whether the PCR reaction is successful**. Thus, negative results for HIV-1 detection from our clinical samples are most likely the result of the absence of virus. [Ex 1012, p. 293, first full paragraph, emphasis added.]

Murakawa does not point out, and we do not find, where the 1988 Murakawa article describes or suggests using the described internal control as a means for quantitating the actual amount of a target viral nucleic acid sequence in a sample. Rather, the 1988 Murakawa article suggests using HTLVAT7 "to direct specific and efficient T7 RNA polymerase-mediated transcription of that amplified sequences, thus enhancing the sensitivity and simplifying the labor of the experiment" (Ex 1012, abstract). Moreover, Murakawa's reply does not dispute Wang's statement that "the only example in the Murakawa publication in which quantitation is performed does not employ shared primers (see Exhibit 1012, Fig. 4, p. 291)" (Paper 44, p. 22).

Third, according to its abstract, the Cantin '802 patent (Ex 1020) is directed to "[a] method of inhibiting human immunodeficiency virus (HIV) comprising administering a therapeutically effective amount of an antiviral agent to attack the first splice acceptor site of the tat III gene of HIV." Murakawa cites to the following text in the Cantin '802

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patent (complete paragraph presented).

A modified polymerase chain reaction amplification assay was also used in accordance with the present invention to determine the effect of the OMP [i.e., oligodeoxyribonucleoside methylphosphonates<sup>7</sup>] treatment on viral RNA synthesis. Details of this amplification procedure are disclosed in copending application Ser. No. 941,379,<sup>8</sup> filed Dec. 15, 1986 with the exception that a modified version of the HIV 3' ORF region is constructed such that the sequences between the two primer sites are altered in length and composition. Such a modification allows for a control RNA or DNA template of fixed concentration to be made which is included with the experimental RNA or DNA samples. The probe for identifying this altered sequence is different from that used for the HIV samples, thereby enabling differentiation of the template amplification from the HIV sequence amplification. The amount of the altered template remains constant thereby enabling one to determine the ratio of amplification of the altered template versus the authentic HIV templates. [Ex 1020, c. 4, ll. 42-60.]

It is clear that the Cantin '802 patent allows one of ordinary skill in the art to determine whether treatment with an antiviral agent, i.e., OMP, has an effect on viral growth. However, as pointed out by Wang (Paper 44, p. 11, fact 68), "[t]he Cantin '802 patent (Exhibit 1020) does not contain the step of calculating the amount of target RNA in the sample that is found in claim 1 of the Wang '727 patent." Murakawa admits as much argues that "the Cantin '802 patent suggests the calculation of the amount of the target sequence through a comparison with the known amount of reference sequence" (Paper 45, p. 3, ¶ 1). Suggesting a comparison, e.g., making a relative, semi-

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<sup>7</sup> Cantin explains antisense RNA as a naturally occurring regulatory control sequence that directs synthesis of RNA and is complementary to a specific mRNA (Ex 1020, c. 1, ll. 18-26). The OMPs of Cantin are analogs in which a 3'-5' methylphosphonate linkage replaces the phosphodiester linkage found in naturally occurring nucleic acids (id., c. 1, ll. 59-63). Thus, the OMPs maintain the complementarity, i.e., specificity, of an antisense RNA but can be used to inhibit mRNA translation thereby reducing viral DNA and infectious virus production (id., c. 1, l. 64 - c. 2, l. 15; c. 2, ll. 35-57).

<sup>8</sup> According to PTO records, application 06/941,379 is now abandoned.

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quantitative estimation, is not the same thing as calculating the actual amount.

The cited portion of the Cantin '802 patent simply discusses altering the length and composition of "the sequences between the two primer sites." Murakawa has presented no evidence that one of ordinary skill in the art would have understood this to have meant using the same primer pair to amplify simultaneously target and reference sequences in the same PCR reaction. Moreover, according to the Cantin '802 patent the details of the amplification procedure are disclosed in an abandoned application, i.e., application 06/941,370. The Cantin '802 patent does not provide (nor has Murakawa) the details which Murakawa argues "suggest" the invention of Wang '727 claim 1. Instead, Figure 2 of the Cantin '802 patent suggests visually comparing relative signal ratios between various samples. According to the Cantin '802 patent,

[t]he lanes shown in FIG. 2 are as follows: A, in vitro transcript of amplified region with  $10^{-7}$   $\mu$ M concentration of a non-specific OMP complementary to HSV 1 (herpes simplex virus 1) sequences; C and D, positive controls from cells infected with HIV in the absence of OMP treatment; E, sample from HIV infected cells pretreated with OMP-C, the sense sequence; F, sample from HIV infected cells pretreated with OMP-A, the antisense sequence. For each reaction involving HIV infected cells, 1  $\mu$ g of total cellular RNA was used for amplification. After the amplified DNA were subjected to gel electrophoresis, blotted to a nylon filter membrane, and hybridized to detection probes, the filter was exposed to x-ray film for 12 hours to obtain the exposure of FIG. 2. [c. 5, ll. 7-25.]

Based upon the foregoing, proposed Murakawa claim 50, the 1988 Murakawa article and the Cantin '802 patent all appear to relate to determining "relative" amounts of RNA. In other words, reading both the 1988 Murakawa article and the Cantin '802 patent as being directed to detecting the presence or absence of a target RNA sequence relative to a predefined amount of a control or reference nucleic acid

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sequence is entirely consistent with "determining the relative quantification of the target sequence" as recited in proposed claim 50, e.g., whether the target sequence is present or absent in the sample. Moreover, insofar as Murakawa argues that its proposed claim 50 is claim 27 as originally filed (Paper 38, p. 1), we note that original claim 27 ultimately depended on original independent claim 18 which recited "a process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood cell sample" (Ex 1015, p. 3). Thus, original claim 27 also suggests that proposed claim 50 is directed to detecting a target RNA sequence in a method having a minimum level of sensitivity based on the ability to detect an amplified product of a known amount of a reference sequence.

Combining proposed Murakawa claim 50 with the 1988 Murakawa article (Ex 1012) and the Cantin '802 patent (Ex 1020) as suggested by Murakawa does not lead to the invention of Wang '727 claim 1. None of proposed Murakawa claim 50, the 1988 Murakawa article or the Cantin '802 patent, alone or in combination, disclose or suggest determining the actual amount of target sequence by simultaneously amplifying the target nucleic acid sequence and a known amount of an added reference sequence with the same primer pair in a single reaction mixture as recited in Wang '727 claim 1. Obviousness requires a suggestion of all limitations in a claim. In re Royka, 490 F.2d 981, 985, 180 USPQ 580, 583 (CCPA 1974).

#### **B. Conclusion**

Based on the foregoing, the invention of Wang '727 claim 1 is not the same patentable invention as the invention of proposed Murakawa claim 50 because Wang

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'727 claim 1 is neither anticipated by proposed Murakawa claim 50 nor rendered obvious by proposed Murakawa claim 50 in view of the 1988 Murakawa article and the Cantin '802 patent. Specifically, Wang '727 claim 1 is not anticipated by proposed Murakawa claim 50 because proposed claim 50 (a) fails to require, explicitly or inherently, use of a shared primer pair to amplify both target and reference sequences as required by Wang claim 1 and (b) fails to recite a sufficiently small genus of reference sequences that one of ordinary skill in the art would immediately envisage use of shared primer part to amplify both target and reference sequences as required by Wang claim 1. Alternatively, Wang '727 claim 1 is not rendered obvious by the "prior art" because, assuming arguendo that one of ordinary skill in the art would have been motivated to combine proposed Murakawa claim 50, the 1988 Murakawa article and the Cantin '802 patent with a reasonable expectation of success, this "prior art" would have not have suggested calculating the actual amount of target nucleic acid in a sample as required by Wang claim 1. Since Wang '727 claim 1 and proposed Murakawa claim 50 do not define the same patentable subject matter, there is no interference-in-fact between the subject matter of any of the claims of the 1993 and 1995 Wang patents and the subject matter of proposed Murakawa claim 50. Consequently, we need not, and do not reach, whether proposed Murakawa claim 50 is unpatentable under 35 U.S.C. §§ 112, 102, 103 and/or 135(b).



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Appellants' Brief on Appeal Under 37 C.F.R. § 41.37  
dated 25 January 2010

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FINAL JUDGMENT  
INTERFERENCE NO. 105,055  
DATED APRIL 5, 2004

PAPER NO. 51 IN U.S. SERIAL NO. 07/402,450  
(Paper No. 48 in Interference No. 105,055)

The opinion in support of the decision being  
entered today is not binding precedent of the Board.

51  
Paper

By: Trial Section Merits Panel  
Board of Patent Appeals and Interferences  
U.S. Patent and Trademark Office  
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Filed: 5 April 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES  
(Administrative Patent Judge Carol A. Spiegel)

---

ALICE M. WANG, MICHAEL E. DOYLE  
and DAVID F. MARK

Junior Party,  
U.S. Patent 5,219,727  
U.S. Patent 5,476,774

v.

GEORGE MURAKAWA, R. BRUCE WALLACE,  
JOHN A. ZAIA and JOHN J. ROSSI

Senior Party,  
Application 07/402,450

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Patent Interference No. 105,055

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Before: SCHAFFER, TORCZON and SPIEGEL, Administrative Patent Judges.  
SPIEGEL, Administrative Patent Judge.

FINAL JUDGMENT

This interference was declared because an interference-in-fact was thought to

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exist between all the pending claims in Murakawa's application, i.e., Murakawa claims 34-35, 38-39, 42-44 and 46-47, and various claims of the Wang patents (Paper 1). All of Murakawa's claims were held to be barred under 35 U.S.C. § 135(b) by the 1993 Wang U.S. Patent 5,219,727 (Paper 36). Murakawa was given the opportunity to cure its § 135(b) problem by filing a motion to add one (1) claim that interferes with the claimed subject matter of Wang patents 5,219,727 and 5,476,774 and is not time barred by § 135(b) (Paper 37). Murakawa filed Murakawa Preliminary Motion 1 (Paper 38) to add proposed claim 50. We have denied this motion (Paper 47). Thus, the only pending claims in Murakawa's involved application, i.e., Murakawa claims 34-35, 38-39, 42-44 and 46-47, are unpatentable under § 135(b)(1). Section 135(b) was enacted to be "a statute of repose . . . a statute of limitations, so to speak, on interferences so that the patentee might be more secure in his property right." Corbett v. Chisholm, 568 F.2d 759, 765, 196 USPQ 337, 342 (CCPA 1977). See also, In re McGrew, 120 F.3d 1236, 1238, 43 USPQ2d 1632, 1635 (Fed. Cir. 1997) (Noting that § 135(b) acts as a statute of limitation or repose); Berman v. Housey, 291 F.3d 1345, 1348, 63 USPQ2d 1023, 1027 (Fed. Cir. 2002) ("Both the plain language of that provision and the relevant legislative history make clear that it [§ 135(b)] was intended to be a statute of repose, limiting the time during which an interference may be declared 'so that the patentee might be more secure in his property right'", citing Corbett, 568 F.2d at 765, 196 USPQ at 342.) Continuation of this interference under the circumstances of this case would be contrary to the purpose of § 135(b) to act as a statute of limitation or repose. We, therefore, enter judgment against Murakawa.

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Termination of the interference at this point without an ORDER TO SHOW CAUSE ("O.C.") is consistent with securing "the just, speedy, and inexpensive determination" of the interference by avoiding needlessly subjecting the parties to redundant and unnecessary expenditures of time, effort and money. 37 CFR § 1.601. The threshold § 135(b) issue has been fairly presented and fully briefed and decided by a three judge panel. Murakawa has been provided the opportunity to cure its § 135(b) problem. Murakawa Preliminary Motion 1 has also been fairly present, fully briefed and decided by a three judge panel. Hence, an O.C. would provide on process or relief that has not already been provided. Finally, attention is directed to 37 CFR § 1.658(b).

Accordingly, it is

**ORDERED** that judgment on priority as to Counts 1 and 2 (Paper 1, p. 5) is awarded against senior party GEORGE J. MURAKAWA, R. BRUCE WALLACE, JOHN A. ZAIA and JOHN J. ROSSI.

**FURTHER ORDERED** that senior party GEORGE J. MURAKAWA, R. BRUCE WALLACE, JOHN A. ZAIA and JOHN J. ROSSI is not entitled to a patent containing

(i) claims 34-35, 38-39, 42-44 and 46-47 (corresponding to Count 1), and

(ii) claim 45 (corresponding to Count 2)

of application 07/402,450, filed September 1, 1989.

**FURTHER ORDERED** that a copy of this paper shall be made of record in the files of U.S. Patents 5,219,727 and 5,476,774 and of application 07/402,450.

**FURTHER ORDERED** that a copy of the decisions on motions filed November 5,

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2003 (Paper 36) and April 5, 2004 (Paper 47) shall be made of record in the files of  
U.S. Patents 5,219,727 and 5,476,774 and of application 07/402,450.

**FURTHER ORDERED** that if there is a settlement agreement which has not  
been filed, attention is directed to 35 U.S.C. § 135(c) and 37 CFR § 1.661.

  
RICHARD E. SCHAFER  
Administrative Patent Judge

  
RICHARD TOBOZON  
Administrative Patent Judge

  
CAROL A. SPIEGEL  
Administrative Patent Judge

BOARD OF PATENT  
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DECISION ON REHEARING  
INTERFERENCE NO. 105,055  
DATED APRIL 29, 2004

PAPER NO. 50 IN INTERFERENCE NO. 105,055

The opinion in support of the decision being  
entered today is not binding precedent of the Board.

Paper 50

By: Trial Section Merits Panel  
Board of Patent Appeals and Interferences  
U.S. Patent and Trademark Office  
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Filed: 29 April 2004

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS  
AND INTERFERENCES  
(Administrative Patent Judge Carol A. Spiegel)

ALICE M. WANG, MICHAEL E. DOYLE  
and DAVID F. MARK

Junior Party,  
U.S. Patent 5,219,727  
U.S. Patent 5,476,774

v.

GEORGE MURAKAWA, R. BRUCE WALLACE,  
JOHN A. ZAIA and JOHN J. ROSSI

Senior Party,  
Application 07/402,450

Patent Interference No. 105,055

Before: SCHAFFER, TORCZON and SPIEGEL, Administrative Patent Judges,  
SPIEGEL, Administrative Patent Judge.

DECISION ON REHEARING

I. Introduction

Murakawa has filed a request for reconsideration (Paper 49) on the denial of

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Murakawa preliminary motion 1 (Paper 47).

For reasons set forth in the MEMORANDUM OPINION and ORDER (Paper 36) of November 5, 2003, the Board concluded that all of the involved Murakawa claims, i.e., Murakawa claims 34-35, 38-39 and 42-47 are barred under 35 U.S.C. § 135(b)(1) by the 1993 Wang U.S. Patent 5,219,727. Murakawa was ordered to submit one (1) claim that interferes with the claimed subject matter of Wang patents 5,219,727 and 5,476,774 and is not time barred by § 135(b)(1) in order for this interference to continue (Paper 37). Murakawa preliminary motion 1 sought to add proposed Murakawa claim 50 to its involved application and to designate it as corresponding to Count 1. Murakawa preliminary motion 1 was denied because its proposed claim 50 does not interfere with the subject matter of the involved Wang claims. [Paper 47, p. 2.] Since there is no interference-in-fact between the subject matter of any of the claims of the 1993 and 1995 Wang patents and the subject matter of proposed Murakawa claim 50, the Board did not reach "whether proposed Murakawa claim 50 is unpatentable under 35 U.S.C. §§ 112, 102, 103 and/or 135(b)" (*id.*, p. 30).

Final judgment was entered against Murakawa because all the pending claims in Murakawa's involved application were held to be barred by § 135(b) and because Murakawa failed to cure its § 135(b) problem when given the opportunity to do so (Paper 48). In other words, "[c]ontinuation of this interference under the circumstances of this case would be contrary to the purpose of § 135(b) to act as a statute of limitation or repose" (*id.*, p. 2).

## **II. Murakawa's request for reconsideration**

In essence, Murakawa position is that "fairness dictates that the Decision (Paper 47) or the Final Judgment (Paper 48) be modified to direct entry of Murakawa's Amendment containing claim 50 and recommendation that the Examiner consider the patentability of this claim" (Paper 49, p. 8).

According to Murakawa, 37 CFR §§ 1.659 and 1.664(a) give the Board the discretion to enter proposed claim 50 and to return Murakawa's involved application to ex parte prosecution (Paper 49, pp. 1-2 and 6).

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Murakawa is of the opinion that such action is warranted because

(i) the Board did not address the patentability of proposed claim 50 (id., pp. 1 and 6-7);

(ii) it would ensure that all of the issues concerning the patentability of proposed claim 50 would be considered and dealt with by an examiner familiar with the art to which the subject matter of this claim pertains (id., p. 7);

(iii) it is consistent with Congress' direction for further examination of certain applications, i.e., Section 532 of Public Law 103-465 (id., ¶ bridging pp. 7-8);

(iv) "Murakawa is entitled to the transitional continued prosecution rules of 37 C.F.R. § 1.129(a), if appropriate" (id., p. 2);

(v) "[t]he term of any patent issuing from any continuation application has already been seriously eroded by the long delay in the initiation of this interference" (id., p. 2, n.1) and proposed "claim 50 would have interfered with Wang's claims under the one-way patentability test for interference-in-fact that was being applied at the time of Murakawa's request for this interference" (id., p. 1);

(vi) it would "preserve the filing date and thus the 17-year term of any patent that issues from the Murakawa application" (id., p. 2); and,

(vii) it would "conserve the Board's and judicial resources" (id., p. 8).<sup>1</sup>

### III. Reconsideration

#### A. Any claim submitted by Murakawa in an effort to cure its § 135(b) problem had to satisfy the two-way patentability test in order to interfere-in-fact with at least one of Wang's involved claims

Murakawa's request does not specify with particularity any points believed to have been misapprehended or overlooked by the Board in rendering its finding of no interference-in-fact between any of Wang's involved claims and proposed Murakawa claim 50. Instead, Murakawa argues that its proposed claim would have interfered

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<sup>1</sup> "The examination of Murakawa's claim 50 for patentability can best be performed by an examiner familiar with the art to which the subject matter of this claim pertains" (Paper 49, p. 7, ¶ 2).

"Murakawa is willing to abide by the Board's Decision and Judgment and would not challenge the Board's finding of no interference-in-fact by appeal or a civil action, if Murakawa's claim 50 is examined for patentability by the examiner" (id., p. 8, last ¶).

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under the one-way patentability test for interference-in-fact allegedly being applied at the time Murakawa sought to provoke this interference.<sup>2</sup>

Whether one agrees with Murakawa's premise that a one-way patentability test was used to determine interference-in-fact at the time Murakawa is said to have been trying to provoke this interference or not, a view to which we do not subscribe,<sup>3</sup> two facts are clear -- on July 3, 2003, prior to the time that Murakawa was afforded the opportunity to cure its § 135(b) problem (i.e., November 5, 2003 (Paper 37)), our reviewing court held that determination of interference-in-fact requires a two-way analysis. See Eli Lilly & Co. v. Board of Regents of the University, 334 F.3d 1264, 67 USPQ2d 1161 (Fed. Cir. 1993). Thus, any claim submitted by Murakawa in an effort to cure its § 135(b) problem clearly had to satisfy the two-way patentability test to interfere-in-fact with at least one of Wang's involved claims at the time the claim was filed if this interference was to continue. Termination of the interference at this point was consistent with securing "the just, speedy, and inexpensive determination" of the interference by avoiding needlessly subjecting the parties to redundant and unnecessary expenditures of time, effort and money. 37 CFR § 1.601. Similarly, there was no need to expend the limited resources of the Board to decide whether or not non-interfering proposed claim 50 was patentable.

**B. Murakawa is not without recourse if the Board does not exercise its discretion to enter proposed claim 50 and direct the examiner to consider the patentability of claim 50**

Assuming arguendo that the Board has the discretion to enter proposed Murakawa claim 50, we decline to do so for the following reasons. First, Murakawa is not without recourse if it desires entry of proposed claim 50 and consideration of the

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<sup>2</sup> Murakawa did not identify the date on which it request this interference. However, Murakawa's involved application 07/402,450 was filed on September 1, 1989 and was accorded benefit for the purpose of priority of application 07/148,959, filed January 27, 1988, application 07/143,045, filed January 12, 1988.

<sup>3</sup> See Case v. CPC International, Inc., 730 F.2d 745, 221 USPQ 196 (Fed. Cir. 1984); Aelony v. Am. 547 F.2d 566, 192 USPQ 486; Nitz v. Ehrenreich, 537 F.2d 539, 190 USPQ 413 (CC PA 1976).

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patentability of this claim.

**1. public law 103-465, § 532**

As alluded to by Murakawa, legislation implementing the General Agreement on Tariffs and Trade (GATT) Uruguay Round Agreements<sup>4</sup> changed the term of patents issued from applications filed on or after June 8, 1995. Prior to the legislation, the term of a U.S. patent was seventeen years from issue date. Theoretically, a patent application could remain pending indefinitely by refiling continuing applications. After the legislation, patent term begins on the issue date and is for twenty years from the date when the earliest U.S. application was filed.<sup>5</sup> Thus, under the new law where there is a lengthy application process, the term of the patent that is ultimately issued is shortened by the amount of time that has passed from filing to issuance and shortened even further taking into account any reference made in such application to any earlier filed application under 35 U.S.C. 120, 121, and 365(c). However, the legislation provided for a "transitional practice" providing for further examination after final rejection of applications pending for two years or more as of June 8, 1995 as set forth in 37 CFR § 1.129.

**2. 37 CFR § 1.129**

Under 37 CFR § 1.129(a), submissions made after final rejection, including information disclosure statement, an amendment to the written description, claims or drawings and a new substantive argument or new evidence in support of patentability, will be entered into the record if such submissions are made prior to or with a notice of appeal and the requisite fee is paid.<sup>6</sup> An applicant may have only two submissions

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<sup>4</sup> Uruguay Round Agreements Act, Public Law No. 103-465, 108 Stat. 408 (1994) (codified as amended at 19 U.S.C. § 3501 (1995)).

<sup>5</sup> Uruguay Round Agreements Act, Public Law No. 103-465, § 532(a)(1), 108 Stat. at 4983-84 (codified as amended at 35 U.S.C. § 154(a)(2)(1995)).

<sup>6</sup> According to 37 CFR § 1.129(e),  
[a]n applicant in an application, other than for reissue or a design patent, that has been pending for at least two years as of June 8, 1995, taking into account any reference made in such application to any earlier filed application under 35 U.S.C. 120, 121 and 365(c), is entitled to have a first submission entered and considered on the merits after

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entered as a matter of right under Rule 129(a). Moreover, further examination under Rule 129(a) is in fact continued examination of the same application and, therefore, applicant may not switch inventions (divisional equivalent) or add new matter (CIP equivalent) as a matter of right.<sup>7</sup> In other words, Rule 129(a) effectively enables applicant to reopen prosecution of the pending application on two separate occasions by paying a fee for each occasion and to avoid the impact of refiling the application to obtain consideration of additional claims and/or information relative to the claimed subject matter.

Murakawa application 07/402,450, filed September 1, 1989, is a pre-GATT application, i.e., it is a U.S. application pending for two years or more as of June 8, 1995. As noted by Murakawa (Paper 49, p. 2), Murakawa may take advantage of transitional Rule 129(a) practice to the full extent of its availability to Murakawa.<sup>8</sup> Thus, at least one possible alternative open to Murakawa is to submit its proposed claim 50 and information relative under 37 CFR § 1.129(a). A rule 129(a) submission would ensure that all of the issues concerning the patentability of proposed claim 50 would be considered and dealt with by an examiner familiar with the art to which the subject

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final rejection under the following circumstances: The Office will consider such a submission, if the first submission and the fee set forth in § 1.17(r) are filed prior to the abandonment of the application. The finality of the final rejection is automatically withdrawn upon timely filing of the submission and payment of the fee set forth in § 1.17(r). If a subsequent final rejection is made in the application, applicant is entitled to have a second submission entered and considered on the merits after the subsequent final rejection under the following circumstances: The Office will consider such a submission, if the second submission and a second fee set forth in § 1.17(r) are filed prior to the filing of an appeal brief and prior to abandonment of the application. The finality of the subsequent final rejection is automatically withdrawn upon the timely filing of the submission and payment of the second fee set forth in § 1.17(r). Any submission filed after a final rejection made in an application subsequent to the fee set forth in § 1.17(r) having been twice paid will be treated as set forth in § 1.116. A submission used in this paragraph includes, but is not limited to, an information disclosure statement, an amendment to the written description, claims or drawings and a new substantive argument or new evidence in support of patentability.

<sup>7</sup> We take no position on whether proposed claim 50 is directed to the same invention under examination in application 07/402,450.

<sup>8</sup> No record of a submission pursuant to 37 CFR § 1.629(a) was found in a cursory review of Murakawa application 07/402,450.



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